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<p>(21) International Application Number: PCT/US97/13562 (22) International Filing Date: 1 August 1997 (01.08.97) (30) Priority Data: 60/023,033 2 August 1996 (02.08.96) US (71) Applicant: BRISTOL-MYERS SQUIBB COMPANY [US/US]; 345 Park Avenue, New York, NY 10154 (US). (72) Inventors: ROSOK, Mae, Joanne; 6340 N.E. 194th Street, Seattle, WA 98155 (US). YELTON, Dale, E.; 2307 19th Avenue East, Seattle, WA 98112 (US). (74) Agent: ADRIANO, Sarah, B.; Merchant, Gould, Smith, Edell, Welter & Schmidt, Suite 400, 11150 Santa Monica Boulevard, Los Angeles, CA 90025 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>																								
<p>(54) Title: A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS</p> <div data-bbox="467 1171 1010 1486"><table border="1"><caption>Estimated data from plasma clearance graph</caption><thead><tr><th>Hours</th><th>chimeric BR96 [serum Ab] (µg/ml)</th><th>cBR96-A [serum Ab] (µg/ml)</th></tr></thead><tbody><tr><td>0</td><td>~800</td><td>~800</td></tr><tr><td>25</td><td>~400</td><td>~100</td></tr><tr><td>50</td><td>~200</td><td>~30</td></tr><tr><td>75</td><td>~100</td><td>~10</td></tr><tr><td>100</td><td>~60</td><td>~5</td></tr><tr><td>150</td><td>~30</td><td>~1</td></tr><tr><td>200</td><td>~10</td><td>~0.5</td></tr></tbody></table></div> <p>Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.</p> <p>(57) Abstract</p> <p>The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject comprising administering an immunoglobulin or Ig fusion protein molecule to the subject, the immunoglobulin molecule having a variable region and a constant region, the immunoglobulin molecule being modified prior to administration by inactivation of at least a portion of the constant region.</p>			Hours	chimeric BR96 [serum Ab] (µg/ml)	cBR96-A [serum Ab] (µg/ml)	0	~800	~800	25	~400	~100	50	~200	~30	75	~100	~10	100	~60	~5	150	~30	~1	200	~10	~0.5
Hours	chimeric BR96 [serum Ab] (µg/ml)	cBR96-A [serum Ab] (µg/ml)																								
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5 **A METHOD FOR INHIBITING IMMUNOGLOBULIN-INDUCED
TOXICITY RESULTING FROM THE USE OF IMMUNOGLOBULINS IN
THERAPY AND IN VIVO DIAGNOSIS**

10 Throughout this application various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

15 **TECHNICAL FIELD OF THE INVENTION**

The present invention relates to methods for inhibiting or reducing immunoglobulin-induced toxicity resulting from therapy or in vivo diagnosis. Specifically, in lieu of using unmodified antibodies or recombinant binding proteins for in vivo use, the
20 invention provides the use of modified antibodies or recombinant binding proteins which have been structurally altered in the constant domain so that upon administration immunoglobulin-induced toxicity is reduced or inhibited.

BACKGROUND OF THE INVENTION

25 Over the years investigators have attempted to harness the immune system for therapeutic use. Immunoglobulin (Ig) molecules which constitute an important part of the immune system are of great interest because they (1) react with a diverse family of ligands, (2) possess different effector functions and (3) are of great
30 biological importance. Despite its potential, a persistent problem with

immunoglobulin immunotherapy has been, among other problems, the toxic effect to normal cells of using antibodies which recognize both normal and diseased cells. This problem is far-reaching because the majority of antibodies presently available recognize a target located on both normal and diseased cells (Slavin-Chiorini, et al.,
5 Int. J. Cancer 53: 97-103 (1993)).

The constant region can promote cell death through antibody dependent cell mediated cytotoxicity (ADCC) or by complement dependent cytotoxicity (CDC). Despite the deletion of portions of the constant region, particularly the CH₂ domain,
10 the antigen binding function can be retained (D. Yelton, M. Scharf, Mutant monoclonal antibody with alterations in biological functions, J. Exp. Methods 156:1131-1148 (1982)).

Others have generated a CH₂-deleted antibody (Mueller et al., Proc. Natl. Acad. Sci.
15 USA 87: 5702-5705 (1990)). Their findings provide that the CH₂-deleted antibody was cleared from the blood of tumor-bearing mice much faster than the corresponding intact antibody. Other in vivo findings also confirmed that a CH₂-deleted antibody, designated ch14.18DCH2, is a potentially useful reagent for radioimmunodetection of human tumors because of its reduced immunogenicity,
20 increased target specificity, and rapid clearance from circulation (Mueller et al., Proc. Natl. Acad. Sci. USA 87: 5702-5705 (1990)).

Generally, whole antibody molecules are composed of two heavy (H) and two light (L) chains which are held together by covalent bonds (disulfide) and non-covalent
25 interactions. Each chain contains a variable region (V) and a constant region (C). The variable regions at the amino termini of the two chains form the antigen binding region. The constant region of the H chain has three components or domains. Occasionally, the first constant region domain (CH₁) interacts with the C region of the L chain through hydrophobic interactions and generally a disulfide bond,

depending on isotype. The next C region stretch is the hinge-acting disulfide bond stably introduced between two H chains. The second constant region domain (CH₂) is adjacent to the hinge region. CH₂ contains sequences important for effector functions of the antibody, such as the sequences responsible for complement
5 fixation, and Fc receptor binding. The third constant region domain (CH₃) is located at the carboxyl terminus of the H chain, and is considered to play an important role in H chain assembly as well as some C region functions.

Today many antibodies in clinical trials are directed against tumor associated
10 antigens. Most tumor associated antigens are not tumor specific but are also generally found on the cell surface of some normal, non-tumorigenic cells. The clinical use of some antibodies directed against tumor associated antigens are limited because of the toxicity associated with their use. Therefore, there is a need for methods for inhibiting toxicity associated with immunoglobulin use in the field of
15 disease therapy (e.g., therapy for tumors, kidney disease, and the like) and in vivo diagnosis.

We addressed this need by discovering methods for inhibiting or reducing toxicity to normal cells generally associated with immunoglobulin immunotherapy or in vivo
20 diagnosis, wherein the immunoglobulin recognizes both diseased and normal cells. Our discovery involves generating immunoglobulin molecules or Ig fusion proteins having structurally altered constant regions which inhibit or reduce immunoglobulin-induced toxicity.

25 SUMMARY OF THE INVENTION

The present invention provides methods for inhibiting immunoglobulin-induced toxicity by using known immunoglobulin or Ig fusion protein molecules which are structurally altered in their constant regions so that the resulting structurally altered

immunoglobulin or Ig fusion protein molecules exhibit reduced or inhibited toxicity in vivo compared to their original unmodified counterparts.

Structural alteration of the constant region may be effected in a number of ways as long as it results in reducing or inhibiting immunoglobulin-induced toxicity.

In accordance with the practice of the invention, structural alteration of the constant region is effected by deletion of the entire constant region. In another embodiment, only the CH₂ domain is deleted. In another embodiment, only that portion of the CH₂ domain that binds the Fc receptor is deleted. In yet another embodiment, only that portion of the CH₂ domain that binds the complement component C1q is deleted. Alternatively, in another embodiment, multiple deletions in discrete Fc receptor and complement component binding domains are effected.

Alternatively, structural alteration is effected by single or multiple mutations in the CH₂ domain such as amino acid insertions and substitutions. The mutation or mutations must result in inhibiting immunoglobulin-induced toxicity. By way of example, the amino acids in multiple toxicity associated domains in the constant region can be altered so as to render the constant region unable to mediate a ADCC response or activate complement thereby inhibiting immunoglobulin induced toxicity resulting from immunotherapy. Alternatively, multiple amino acids in a single toxicity associated domain in the constant region can be altered.

Further alternatively, structural alteration can be effected by isotype switching resulting in an altered immunoglobulin molecule that either does not induce toxicity or induces some limited toxicity but does not cause a harmful effect. For example, isotype switching can result in the constant region being unable to mediate a CDC or ADCC response or some other activity which mediates toxicity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a line graph showing plasma clearance in high Le^y expressing dogs using chimeric BR96 versus constant region mutant of cBR96-2.

5

Figure 2 is a schematic diagram of a plasmid designated pTWD-cJVK.L1 including the chimeric (c)BR96-light chain (SEQ ID NO. 11).

10 Figure 3 is a schematic diagram of a plasmid designated pD16hJ1.L1 including the human (h)BR96-light chain (SEQ ID NO. 13).

Figure 4 is a schematic diagram of a plasmid, designated pD17-hJm14-dCH2.H1, of hBR96-2A (i.e., human mutant BR96 having the H1, H2, and H3 mutations and the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

15

Figure 5 is a schematic diagram of a plasmid, designated pD17-cJ-dCH2.H1, of cBR96-A (SEQ ID NO. 10) (i.e., chimeric BR96 having the CH₂ deletion (PCT Application No. 95/305444, published March 6, 1996)).

20 Figure 6 is a schematic diagram of a plasmid, designated pD17-cJ.H1, of cBR96.

Figure 7 is a line graph showing the results of an ELISA assay of (1) hBR96-2A-Dox to Le^y (closed diamond), (2) hBR96-2A to Le^y (96:0006A2 R/A)(closed square), (3) hBR96-2A to Le^y (96:0006B R/A)(closed triangle), and BR96-Dox to
25 Le^y (X).

Figure 8 is a line graph showing the results of an ELISA assay of (1) BR96-A-Dox to Le^y (closed diamond), (2) chiBR96 to Le^y (closed square), (3) cBR96-A to Le^y (96:0003 R/A)(closed triangle), and cBR96-Dox to Le^y (X).

Figures 9a-c are schematic diagrams showing the steps for deleting a CH₂ domain.

Figures 10a-c are schematic diagrams showing the construction of BR96 IgG1 CH₂
5 domain point mutations.

Figure 11 is a schematic diagram showing the construction of the pNg1/14 vector.

Figure 12 is a schematic diagram showing the construction of pD17-hBR96-2.
10

Figure 13 is a schematic diagram showing the construction of pD17-hJm14-
dCH2.H1.

Figure 14 is the nucleic acid sequence of pD17-cJ-dCH2.H1, the plasmid shown in
15 Figure 5, chimeric BR96 having the CH₂ deletion.

Figure 15 is a line graph showing the results of an ELISA assay comparing whole
chiBR96 and deleted CH₂ chiBR96 on Le^y.

20 Figure 16 is a description of the seven structural alterations.

Figure 17 is a schematic diagram of a plasmid designated pD17-hG1b.

Figure 18 is the nucleic acid sequence of pD17-hJm14.H1.
25

Figure 19 is the nucleic acid sequence of pD17-hG1b.

Figure 20 is a line graph showing complement dependent cytotoxicity. In the
legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is

hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b mAb, negative control.

- 5 Figure 21 is a line graph showing antibody dependent cell-mediated cytotoxicity. In the legend, the closed square is hBR96-1; closed diamond is hBR96-2B; closed circle is hBR96-2C; closed triangle is hBR96-2D; open square is hBR96-2H; open circle is hBR96-2A and open triangle is 2B8, anti-*Pseudomonas aeruginosa* flagella type b monoclonal antibody (mAb), negative control.

10

- Figure 22 is a line graph showing binding activity of hBR96-2 constant region mutants on LeY-HSA. In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

15

- Figure 23 is a line graph showing binding activity of hBR96-2 constant region mutants on LNFPIII-BSA. LNFPIII is a lacto-N-fucopentasose, a Lewis X trisaccharide with an additional lactose spacer (V Labs, Covington, LA). In the legend, the solid diamond is hBR96-1; solid square is hBR96-2A (CH2 deletion); solid triangle is hBR96-2B (235, 237 mutations); open square is hBR96-2C (318, 320, 322 mutations); open circle is hBR96-2D (331 mutation); and open triangle is hBR96-2H (235, 237, 318, 320, 322, 331 mutations).

20

- 25 Figures 24A and 24B provide a strategy for introducing multiple mutations by RPCR. (A) Diagram of the 1.4 kpb IgG heavy chain region showing the hinge CH₂ and CH₃ domains as boxed regions. Site-specific mutations to be introduced into CH₂ positions L1, L2, and L3 are encoded by complementary sets of mutant PCR

primers (A1 and A2; B1 and B2; and C1 and C2). The asterisks (*) indicate the number of amino acid changes introduced at each L position. The two PCR primers, Rs (Recombination -sense) and Ra (Recombination-antisense), flank the Eco-47-III restriction sites and mediate homologous recombination with vector ends. The 3' ends of the oligonucleotides are represented by arrowheads. (B) A three-way homologous recombination event between fragments RsA2, A1Ra and the linearized vector produces the L1 mutant IgG. Two distally located sets of mutations (L1 and L2) are simultaneously introduced by increasing the number of recombining PCR products as is shown in the four-way recombination of RsA2, A1B1, B1Ra with vector.

Figure 25 is a gel showing Eco-47-III restriction endonuclease analysis of DNAs prepared from colonies generated by multiple PCR fragment RPCR. Lane M: 1kb ladder DNA marker (GIBCO/BRL Life Science Technology). Lanes 1-12: Twelve randomly selected colonies resulting from quadruple homologous recombination events were used to prepare plasmid and digested with Eco47-III. Clones 1, 2, 6 and 9 contain the fully assembled 1.4 kpb insert.

Figure 26 provides the amino acid sequence for hBR96-2 heavy-chain variable region and the human IgG1 constant region.

Figure 27 provides the amino acid sequence for hBR96-2A heavy-chain variable region and the human IgG1 constant region.

Figure 28 provides the amino acid sequence for chi BR96 heavy-chain variable region and the human IgG1 constant region without the CH₂ domain.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

5 As used herein the term "inhibiting immunoglobulin-induced toxicity" means to reduce or alleviate symptoms generally associated with toxicity caused by immunoglobulin or Ig fusion protein therapy, e.g., toxicity mediated by effector functions of the Fc receptor. For example, BR96 antibody recognizes and binds BR96 antigen which is found at some levels in the gastrointestinal tract and at
10 elevated levels in tumors (as compared to the gastrointestinal tract of normal tissues). The binding of BR96 antibody to BR96 antigen in vivo causes symptoms associated with gastrointestinal toxicity. These symptoms include rapid onset of vomiting, often with blood, and nausea. In humans the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach.

15 The pathology of the wound is limited and resolves. However, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity. For highly elevated levels of other antigens found in the central nervous system (CNS), liver, and other locations, the toxicity will be characterized by
20 symptoms other than those described above.

As used herein the term "immunoglobulin molecule" can be produced by B cells or be generated through recombinant engineering or chemical synthetic means. Examples of immunoglobulin molecules include (1) antibodies, e.g., polyclonal and
25 monoclonal antibodies, chimeric or humanized, and (2) recombinant Ig containing binding proteins, e.g., Ig fusion proteins. Recombinant Ig containing binding proteins include cell surface proteins, e.g., CD antigens (in one embodiment, CTLA4), to which an Ig tail is joined.

As used herein the terms "structurally altered" or "structural alteration" means manipulating the constant region so that the resulting molecule or protein exhibits a diminished ability to induce toxicity. Structural alteration can be by chemical modification, proteolytic alteration, or by recombinant genetic means. Recombinant
5 genetic means may include, but is not limited to, the deletion, insertion and substitution of amino acid moieties.

As used herein the terms "multiple toxicity associated domains" means more than one discrete toxicity associated domain. As there appear to be at least two toxicity
10 associated domains in the immunoglobulin molecule, one roughly localized to amino acids 231-238 and another roughly localized to amino acids 310-331, an example of the structural alteration of multiple toxicity associated domains comprises the insertion, substitution or deletion of amino acid residues in both of these domains. This definition excludes structural alterations targeting a single toxicity associated
15 domain.

Merely by way of example, the constant region of the immunoglobulin molecule can be structurally altered so that the molecule no longer mediates a CDC or ADCC response. However, the methods of the invention encompasses the use of
20 structurally altered immunoglobulin molecules regardless of whether it mediates a CDC or ADCC response. The underlying requirement is that the altered molecule must inhibit immunoglobulin-induced toxicity.

Structural alteration can be effected in a number of ways. For example, structural
25 alteration can be effected by deletion of the entire constant region.

Alternatively, structural alteration can be effected by deletion of the entire CH₂ domain of the constant region. In this instance, deletion of the entire CH₂ domain may render the molecule unable to (1) bind an Fc receptor thereby eliminating the

molecule's possibility of mediating antibody-dependent cellular cytotoxicity (ADCC), (2) bind C1q, or (3) activate complement.

Alternatively, structural alteration can be effected by deletion of only that portion of the CH₂ domain that binds the Fc receptor or complement.

Further alternatively, a single mutation or multiple mutations such as substitutions and insertions in the CH₂ domain can be made. The underlying requirement of any mutation is that it must inhibit, diminish, or block immunoglobulin-induced toxicity.

For example, this can be achieved by mutating the constant region such that the altered molecule is rendered unable to mediate a CDC response or an ADCC response, or to activate complement.

Alternatively, structural alteration can be effected by isotype switching (also known as class switching) so that the altered molecule does not induce toxicity in the subject. In one embodiment, the constant region of the immunoglobulin is structurally altered so that it no longer binds the Fc receptor or a complement component, e.g., switching a molecule's original IgG isotype from IgG1 to IgG4. Isotype switching can be effected regardless of species, i.e., an isotype from a non-human being can be switched with an isotype from a human being (E.D. Finkelman et al. (1990) *Annu. Rev. Immunol.* 8:303-333; T. Honjo et al. (1979) *Cell* 18: 559-568; T. Honjo et al. In "Immunoglobulin Genes" pp. 124-149 Academic Press, London)).

As used herein the term "Ig fusion protein" means any recombinantly produced antigen or ligand binding domain having a constant region which can be structurally altered.

As used herein "cytotoxic agent" includes antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents. Specific examples within these groups include but are not limited to ricin, doxorubicin, daunorubicin, taxol, ethidium bromide, mitomycin, etoposide, 5 tenoposide, vincristine, vinblastine, colchicine, supporin, gelonin, PE40, bryodin, dihydroxy anthracin dione, actinomycin D, and 1-dehydrotestosterone.

As used herein the term "BR96" refers to (1) the whole BR96 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, (2) chimeric BR96 10 monoclonal antibody disclosed in PCT No. 95/305444, published March 6, 1996, or (3) BR96 mutant molecules disclosed in PCT No. 95/305444, published March 6, 1996.

As used herein, "treating" means to (1) provide tumor regression so that the tumor is 15 not palpable for a period of time (standard tumor measurement procedures may be followed (A.B. Miller et al. "Reporting results of cancer treatment" Cancer 47:207-214 (1981)); (2) stabilize the disease; or (3) provide any clinically beneficial effects.

As used herein, an "effective amount" is an amount of the antibody, 20 immunoconjugate, or recombinant molecule which kills cells or inhibits the proliferation thereof.

As used herein, "administering" means oral administration, administration as a suppository, topical contact, intravenous, intraperitoneal, intramuscular or 25 subcutaneous administration, or the implantation of a slow-release device such as a miniosmotic pump, to the subject.

As used herein, "pharmaceutically acceptable carrier" includes any material which when combined with the antibody retains the antibody's specificity or efficacy and is

non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Other carriers may also include sterile solutions, tablets including
5 coated tablets and capsules.

Typically such carriers contain excipients such as starch, milk, sugar, certain types of clay, gelatin, stearic acid or salts thereof, magnesium or calcium stearate, talc, vegetable fats or oils, gums, glycols, or other known excipients. Such carriers may
10 also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods.

As used herein, "mutation" means a single amino acid or nucleic acid mutation or multiple mutations by whatever means, e.g., homologous recombination, error prone
15 PCR, or site directed mutagenesis.

In order that the invention herein described may be more fully understood, the following description is set forth.

20 METHODS OF THE PRESENT INVENTION

The present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from the use of immunoglobulin during therapy or in vivo diagnosis. For example, the methods of the invention would be useful to minimize
25 the toxicity associated with prolonged clinical exposure to immunoglobulin use during or after tumor imaging with radiolabeled antibodies.

In accordance with the practice of this invention, the subject includes, but is not limited to, human, equine, porcine, bovine, murine, canine, feline, and avian

subjects. Other warm blooded animals are also included in this invention.

This method comprises administering an immunoglobulin molecule to the subject. The immunoglobulin can be IgG, IgM, or IgA. IgG is preferred.

5

In one embodiment of the invention, the immunoglobulin molecule recognizes and binds Le^y. In another embodiment, the immunoglobulin recognizes and binds Le^x. In a further embodiment, the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma deposited on February 22, 1989 with the American Type
10 Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852 and
accorded ATCC Accession No.: HB 10036. In yet another embodiment, the
immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma
deposited on May 23, 1990, with the ATCC, 12301 Parklawn Drive, Rockville, MD
20852 and accorded ATCC Accession No.: HB 10460.

15

In accordance with the practice of the invention, the immunoglobulin can be a
bispecific antibody with a binding specificity for two different antigens, one of the
antigens being that with which the monoclonal antibody BR96 produced by the
hybridoma having the identifying characteristics of HB 10036 as deposited with the
20 ATCC binds. Also, in accordance with the practice of the invention, the
immunoglobulin can be an anti-idiotypic antibody.

As required by the invention, at least a portion of the constant region of the
immunoglobulin molecule is structurally altered. Structural alteration can be
25 effected by a number of means. In one embodiment, the entire constant region, i.e.,
CH₁, CH₂, and CH₃ domains, can be deleted.

In another embodiment, only the CH₂ domain is deleted from the immunoglobulin
molecule (e.g., cBR96-A (Figure 5), hBR96-2A (Figure 4). In this embodiment, the

CH₂ deletion may result in a molecule unable to bind the Fc receptor or a complement component.

In another embodiment, only that portion of the CH₂ domain which binds the complement component C1q is deleted. In yet another embodiment, mutations in specific portions of the CH₂ domain are made. For example, the immunoglobulin molecule may be modified by structurally altering multiple toxicity associated domains in the constant region so that immunoglobulin-induced toxicity is inhibited. A discussion of such mutations are further found hereinafter.

10

Regardless of the means, the underlying requirement for any structural alteration of the constant region is that immunoglobulin-induced toxicity is substantially reduced or inhibited. In one embodiment, immunoglobulin-induced toxicity is inhibited by structurally altering the constant region such that the molecule's ability to mediate a CDC response or ADCC response and/or activate the complement cascade is prevented or inhibited. Methods for determining whether the molecule is able to inhibit a CDC response are well known, e.g., one method involves a ⁵¹Cr-release test (H. Garrigues et al. Int. J. Cancer 29:511 (1982); I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to inhibit an ADCC response are well known (I. Hellström et al. PNAS 82:1499 (1985)). Methods for determining whether the molecule is able to activate a complement cascade are well known.

15

In another embodiment of the invention, the method comprises administering to the subject an Ig fusion protein having a structurally altered constant region. Structural alteration of the constant region may include deletion of the entire C region or portions thereof, e.g., alteration of the CH₂ domain so that the altered molecule no longer binds the Fc receptor or a complement component.

20

The invention further provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy in a subject. The method comprises administering to the subject an antibody which has been modified so that at least a portion of the constant region has been structurally altered as discussed supra. In one
5 embodiment, the antibody recognizes and binds Le^y. In another embodiment, the antibody recognizes and binds to Le^x.

In accordance with the practice of this invention, the antibody can be monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of
10 HB 10036 as deposited with the ATCC. Alternatively, the antibody can be chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC. Further, the antibody can be a bispecific antibody with a binding specificity for two different antigens, one of the antigens being that with which the monoclonal antibody BR96 produced by the hybridoma
15 having the identifying characteristics of HB 10036 as deposited with the ATCC binds.

Additionally, the present invention provides a method for inhibiting immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a
20 subject. The disease will vary with the antigen sought to be bound. Examples of diseases include but are not limited to immunological diseases, cancer, cardiovascular diseases, neurological diseases, dermatological diseases or kidney disease.

25 This method comprises the following steps. Step one provides selecting an antibody for a target. Generally, the target is associated with the disease and the antibody directed to the target is known. For example, the target can be the BR96 antigen and the antibody selected is BR96.

Step two of this method provides structurally altering the constant region of the antibody so selected so that immunoglobulin induced toxicity is inhibited. Inactivation can include any of the means discussed above. For example, inactivation can be effected by structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected.

Step three of this method provides administering the structurally altered antibody of step two to the subject under conditions that the structurally altered antibody recognizes and binds the target and that such binding directly or indirectly alleviates symptoms associated with the disease.

In accordance with the invention, in one embodiment step one provides selecting an Ig fusion protein for a target. Further, the method provides mutating the Ig fusion protein so selected by structurally altering the CH₂ domain of the constant region of the Ig protein by the same means discussed above.

The invention further provides methods to treat human carcinoma. For example, the immunoglobulin, antibody, or Ig fusion protein discussed above can be used in combination with standard or conventional treatment methods such as chemotherapy, radiation therapy or can be conjugated or linked to a therapeutic drug, or toxin, as well as to a lymphokine or a tumor-inhibitory growth factor, for delivery of the therapeutic agent to the site of the carcinoma.

Techniques for conjugating therapeutic agents to immunoglobulins are well known (see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellström et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In

Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62:119-58 (1982)).

5

Alternatively, the structurally altered antibody or Ig fusion protein can be coupled to high-energy radiative agents, e.g., a radioisotope such as ^{131}I ; which, when localized at the tumor site, results in a killing of several cell diameters (see, e.g., Order, "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985)).

10 According to yet another embodiment, the structurally altered BR96 antibody can be conjugated to a second antibody to form an antibody heteroconjugate for the treatment of tumor cells as described by Segal in United States Patent 4,676,980.

15

Still other therapeutic applications for the structurally altered antibody or Ig fusion protein of the invention include conjugation or linkage, e.g., by recombinant DNA techniques or protein chemical techniques, to an enzyme capable of converting a prodrug into a cytotoxic drug and the use of that antibody-enzyme conjugate in combination with the prodrug to convert the prodrug to a cytotoxic agent at the tumor site (see, e.g., Senter et al., "Anti-Tumor Effects Of Antibody-alkaline Phosphatase", Proc. Natl. Acad. Sci. USA, 85:4842-46 (1988); "Enhancement of the in vitro and in vivo Antitumor Activities of Phosphorylated Mitomycin C and Etoposide Derivatives by Monoclonal Antibody-Alkaline Phosphatase Conjugates",

20 Cancer Research 49:5789-5792 (1989); and Senter, "Activation of Prodrugs by Antibody-Enzyme Conjugates: A New Approach to Cancer Therapy," FASEB J. 4:188-193 (1990)).

25

It is apparent therefore that the present invention encompasses pharmaceutical compositions including immunoglobulin molecules, antibodies, and Ig fusion proteins all having structurally altered CH₂ domains, and their use in methods for treating human carcinomas. For example, the invention includes pharmaceutical compositions for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of a structurally altered BR96 and a pharmaceutically acceptable carrier.

The compositions may contain the structurally altered antibody or Ig fusion protein or antibody fragments, either unmodified, conjugated to a therapeutic agent (e.g., drug, toxin, enzyme or second antibody). The compositions may additionally include other antibodies or conjugates for treating carcinomas (e.g., an antibody cocktail).

The compositions of the invention can be administered using conventional modes of administration including, but not limited to, intrathecal, intravenous, intraperitoneal, oral, intralymphatic or administration directly into the tumor. Intravenous administration is preferred.

The composition of the invention can be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The compositions of the invention also preferably include conventional pharmaceutically acceptable carriers and adjuvants known in the art such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate.

In accordance with the practice of the invention, the pharmaceutical carrier can be a lipid carrier. The lipid carrier can be a phospholipid. Further, the lipid carrier can be a fatty acid. Also, the lipid carrier can be a detergent. As used herein, a detergent
5 is any substance that alters the surface tension of a liquid, generally lowering it.

In one example of the invention, the detergent can be a nonionic detergent. Examples of nonionic detergents include, but are not limited to, polysorbate 80 (also known as Tween 80 or (polyoxyethylenesorbitan monooleate), Brij, and Triton (for
10 example Triton WR-1339 and Triton A-20).

Alternatively, the detergent can be an ionic detergent. An example of an ionic detergent includes, but is not limited to, alkyltrimethylammonium bromide.

15 Additionally, in accordance with the invention, the lipid carrier can be a liposome. As used in this application, a "liposome" is any membrane bound vesicle which contains any molecules of the invention or combinations thereof.

The most effective mode of administration and dosage regimen for the compositions
20 of this invention depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician.

The interrelationship of dosages for animals of various sizes and species and humans based on mg/m^2 of surface area is described by Freireich, E.J., et al. Cancer
25 Chemother., Rep. 50 (4): 219-244 (1966). Adjustments in the dosage regimen can be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses can be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses can be

administered daily or proportionally reduced depending on the specific therapeutic situation).

THE MOLECULES OF THE INVENTION

5

The present invention provides structurally altered BR96 or BR96 Ig fusion proteins.

Structurally altered BR96 antibodies or Ig fusion proteins have the variable region of BR96 and a modified constant region. This modification provides structurally altered BR96 antibodies or Ig fusion proteins with the ability to inhibit
10 immunoglobulin-induced toxicity.

Various embodiments of structurally altered BR96 or BR96 Ig fusion proteins have been made.

15 In one embodiment, designated cBR96-A, the entire CH₂ domain of cBR96 was deleted. CBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 10. cBR96 is expressed by a plasmid having the sequence in SEQ ID NO. 9.

20 In another embodiment, designated hBR96-2A, the entire CH₂ domain of hBR96 was deleted. hBR96-A is expressed by the plasmid having the sequence shown in SEQ. ID. NO. 12. hBR96 is a mutant BR96 having the H1, H2, and H3 mutations described in PCT Application No. 95/305444, published March 6, 1996.

25 In yet another embodiment, designated hBR96-2B, the leucine residue located at amino acid position 235 is mutated to alanine. Additionally, the glycine residue located at amino acid position 237 is mutated to alanine. The amino acid position numbering used is described in Kabat et al. Sequences of Proteins of Immunological Interest 5th Edition (1991) United States Department of Health and Human Services.

In a further embodiment, designated hBR96-2C, the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine
5 using standard protocols (Alexander R. Duncan and Greg Winter "The binding site for C1q on IgG" Nature 332:738 (1988)).

In another embodiment, designated hBR96-2D, the proline residue at position 331 is mutated to alanine (M-H. Tao et al., "Structural features of human immunoglobulin
10 G that determine isotype-specific differences in complement activation" J. Exp. Med. 178:661-667 (1993); Y. Xu et al., "Residue at position 331 in the IgG1 and IgG4 domains contributes to their differential ability to bind and activate complement" J. Biol. Chem. 269:3469-3474 (1994)).

15 In an additional embodiment, designated hBR96-2E, the leucine residue at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; and the lysine residue located at position 322 is mutated to serine (A. Morgan et al., "The N-terminal end
20 of the CH₂ domain of chimeric human IgG1 anti-HLA-DR is necessary for C1q, Fc(gamma)RI and Fc(gamma)RIII binding" Immunol. 86:319-324 (1995)).

In yet a further embodiment, designated hBR96-2F, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is
25 mutated to alanine; and the proline residue located at position 331 is mutated to alanine.

In yet another embodiment, designated hBR96-2G, the glutamic acid residue located at position 318 is mutated to serine; the lysine residue located at position 320 is

mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

5 In another embodiment, designated hBR96-2H, the leucine residue located at position 235 is mutated to alanine; the glycine residue located at position 237 is mutated to alanine; the glutamic acid residue at position 318 is mutated to serine; the lysine residue located at position 320 is mutated to serine; the lysine residue located at position 322 is mutated to serine; and the proline residue located at position 331 is mutated to alanine.

10

Depending on its form, a structurally altered BR96 antibody or fusion protein can be a monofunctional antibody, such as a monoclonal antibody, or bifunctional antibody, such as a bispecific antibody or a heteroantibody. The uses of structurally altered BR96, i.e., as a therapeutic or diagnostic agent, will determine the different forms of
15 structurally altered BR96 which is made.

Several options exists for antibody expression. Immunoexpression libraries can be combined with transfectoma technology, i.e., the genes for the Fab molecules derived from the immunoglobulin gene expression library can be connected to the
20 desired constant-domain exons. These recombinant genes can then be transfected and expressed in a transfectoma that would secrete an antibody molecule.

Once produced, the polypeptides of the invention can be modified, i.e., by amino acid modifications within the molecule, so as to produce derivative molecules. Such
25 derivative molecules would retain the functional property of the polypeptide, namely, the molecule having such substitutions will still permit the binding of the polypeptide to the BR96 antigen or portions thereof.

It is a well-established principle of protein chemistry that certain amino acid

substitutions, entitled "conservative amino acid substitutions," can frequently be made in a protein without altering either the conformation or the function of the protein.

- 5 Amino acid substitutions include, but are not necessarily limited to, amino acid substitutions known in the art as "conservative".

Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid
10 (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa.

Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional
15 structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine and valine (V).

Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R)
20 are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

- 25 In one embodiment of the present invention, the polypeptide is substantially pure, i.e., free of other amino acid residues which would inhibit or diminish binding of the polypeptide to its target and would inhibit or reduce gastrointestinal toxicity which are normally exhibited during or after antibody therapy.

NUCLEIC ACID MOLECULES ENCODING THE PRESENT INVENTION

The nucleotide sequences and the amino acid sequences of the variable and constant regions of BR96 are known. The sequence for the immunoglobulin constant region
5 is known and provided in Figure 18. Specific mutations in the constant region of the BR96 antibody were made. Nucleic acid molecules encoding the seven mutants described above (hBR96-2B through hBR96-2H) are as follows.

In hBR96-2B, alanine at amino acid positions 235 and 237 is encoded by codons
10 GCU, GCC, GCA, or GCG.

In hBR96-2C, serine at positions 318, 320, and 322 is encoded by UCU, UCC, UCA, or UGG.

15 In hBR96-2D, alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2E, alanine at positions 235 and 237 is encoded by codons GCU, GCC, GCA, or GCG. Serine at positions 318, 320, and 322 is encoded by UCU, UCC,
20 UCA, or UGG.

In hBR96-2F, alanine at positions 235, 237, and 331 is encoded by codons GCU, GCC, GCA, or GCG.

25 In hBR96-2G, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG. Further, the alanine at position 331 is encoded by codons GCU, GCC, GCA, or GCG.

In hBR96-2H, alanine at positions 235, 237, and 331 is encoded by codons GCU,

GCC, GCA, or GCG. Additionally, serine at positions 318, 320, 322 is encoded by UCU, UCC, UCA, or UGG.

Any of the above can be deoxyribonucleic acid (DNA), e.g., complementary DNA
5 (cDNA), or ribonucleic acid (RNA).

IMMUNOCONJUGATES

Immunoconjugates (having whole antibody or Ig fusion proteins) may be
10 constructed using a wide variety of chemotherapeutic agents such as folic acid and anthracyclines (Peterson et al., "Transport And Storage Of Anthracyclines In Experimental Systems And Human Leukemia", in Anthracycline Antibiotics In Cancer Therapy, Muggia et al. (Eds.), p. 132 (Martinus Nijhoff Publishers (1982); Smyth et al., "Specific Targeting of Chlorambucil to Tumors With the Use of
15 Monoclonal Antibodies", J. Natl. Cancer Inst., 76:503-510 (1986)), including doxorubicin (DOX) (Yang and Reisfeld "Doxorubicin Conjugated with a Monoclonal Antibody Directed to a Human Melanoma-Associated Proteoglycan Suppresses Growth of Established Tumor xenografts in Nude Mice PNAS (USA)" 85:1189-1193 (1988)), Daunomycin (Arnon and Sela "In Vitro and in vivo Efficacy
20 of Conjugates of Daunomycin With Anti-Tumor Antibodies" Immunol. Rev., 65:5-27 (1982)), and morpholinodoxorubicin (Mueller et al., "Antibody Conjugates With Morpholinodoxorubicin and Acid-Cleavable Linkers", Bioconjugate Chem., 1:325-330 (1990)).

25 BR96 has been conjugated to doxorubicin and has been shown to be effective in therapy of certain cancers or carcinomas (Trail, P.A., Willner, D., Lasch, S.J., Henderson, A.J., Casazza, A.M., Firestone, R.A., Hellström, I., and Hellström, K.E. Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates. Science, 261:212-215, 1993).

In accordance with the practice of the invention, structurally altered BR96 can be used in forms including unreduced IgG, reduced structurally altered IgG, and fusion proteins (PCT Application No. 95/305444, published March 6, 1996).

5

Suitable therapeutic agents for use in making the immunoconjugate includes Pseudomonas exotoxin A (PE) in either the native PE or LysPE40 form. LysPE40 is a truncated form containing a genetically modified amino terminus that includes a lysine residue for conjugation purposes. Doxorubicin is also a suitable therapeutic

10 agent.

Additional examples of therapeutic agents include, but are not limited to, antimetabolites, alkylating agents, anthracyclines, antibiotics, and anti-mitotic agents.

15

Antimetabolites include methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine.

20

Alkylating agents include mechlorethamine, thiotepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin.

25

Anthracyclines include daunorubicin (formerly daunomycin) and doxorubicin (also referred to herein as adriamycin). Additional examples include mitozantrone and bisantrene.

Antibiotics include dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC).

Antimitotic agents include vincristine and vinblastine (which are commonly referred to as vinca alkaloids).

- 5 Other cytotoxic agents include procarbazine, hydroxyurea, asparaginase, corticosteroids, mytotane (O,P'-(DDD)), interferons.

Further examples of cytotoxic agents include, but are not limited to, ricin, bryodin, gelonin, supporin, doxorubicin, taxol, cytochalasin B, gramicidin D, ethidium

- 10 bromide, etoposide, tenoposide, colchicine, dihydroxy anthracin dione, 1-dehydrotestosterone, and glucocorticoid.

Clearly analogs and homologs of such therapeutic and cytotoxic agents are encompassed by the present invention. For example, the chemotherapeutic agent

- 15 aminopterin has a correlative improved analog namely methotrexate.

Further, the improved analog of doxorubicin is an Fe-chelate. Also, the improved analog for 1-methylnitrosourea is lomustine. Further, the improved analog of vinblastine is vincristine. Also, the improved analog of mechlorethamine is

- 20 cyclophosphamide.

METHODS FOR MAKING MOLECULES OF THE INVENTION

There are multiple approaches to making site specific mutations in the CH₂ domain of an immunoglobulin molecule. One approach entails PCR amplification of the

25 CH₂ domain with the mutations followed by homologous recombination of the mutated CH₂ into the vector containing the desired immunoglobulin, e.g., hBR96-2. For example, hBR96-2B and hBR96-2D have been made by this method.

Another approach would be to introduce mutations by site-directed mutagenesis of single-stranded DNA. For example, vector pD17-hG1b, which contains only the constant region of IgG1 and not the V domain of hBR96, has the fl origin of replication. This gives the vector the properties of a phagemid and site-directed mutagenesis experiments can be performed according to the methods of Kunkel, et al. (Kunkel, T.A., J.D. Roberts, and R.A. Zakour, 1987 Methods Enzymol. 154:367-383) as provided in the Bio-Rad Muta-Gene® phagemid *in vitro* mutagenesis kit, version 2. For example, hBR96-2B, -C, -D, -E, -F, -G, and -H were made by this method.

10

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the scope of this invention in any manner.

15

EXAMPLE 1

The following standard ELISA protocol was used.

- 20 **Materials:** Immulon2 96 well plates and Genetic Systems Specimen Diluent Concentrate (10x); antibody conjugate was Goat Anti Human Kappa-HRP Mouse Adsorbed, Southern Biotech. at 1:10,000 in Genetic Systems Conjugate Diluent (1x); Genetic Systems EIA Chromogen Reagent (TMB) (1:100); Genetic Systems EIA Buffered Substrate (1x); primary antibody or antigen were AffiniPure F(ab')₂ Fragment Goat Anti Human IgG Fc Fragment specific (Jackson Immuno Research),
25 Goat Anti Human Kappa-UNLB (Southern Biotechnology Associates), Le^y-HSA (Alberta Research Council).

Methods: Dilute primary antibody or antigen to 1.0 µg/ml in 0.05M Carb/Bicarb buffer. Add 100µl of the diluted solution per well in Immulon 2 plates. Seal plates and incubate O.N. at 4°C.

- 5 Block plates by flicking them and blotting on paper towels. Add 200µl/well of Genetic Systems, Specimen Diluent Concentrate (1x). Incubate at least 1 hour at room temperature and then dump the contents of the plates. Wash the plates 3x in saline/Tween. Blot to dry. Allow the plates to dry at R.T. (45 min. to 1 hour). Seal and store the plates at 4°C.

10

Test samples as follows. Dilute samples and standards in Specimen Diluent at 1:10. Perform serial dilutions in separate round bottom plates. Transfer 100µl/well of final dilutions to antigen coated assay plates; then incubate O.N. at 4°C. Wash plates 3x with saline/Tween.

15

For conjugation add 100 µl/well of antibody-HRP conjugate in Genetic Systems Conjugate Diluent (1x). Incubate plates at Room Temp. for 60 min. Wash plates 3x in saline/Tween.

- 20 Add 100 µl/well of Genetic Systems EIA Chromogen Reagent (TMB) 1:100 in EIA Buffered Substrate (1x). Incubate at R.T. for 15 min. and stop with 1N H₂SO₄ 100 µl/well. Read plate at 450/630nm in EIA plate reader.

EXAMPLE 2

25

Construction of CH₂ deleted BR96 molecules

Strategy for Deleting CH₂ Domains: To construct CH₂ deleted BR96 molecules, the hinge, CH₂ and CH₃ domains were removed from chimeric BR96 and humanized

BR9696-2 IgG1 molecules by an Eco47-III restriction digestion in non-coding regions. The hinge and CH₃ domains were amplified by polymerase chain reaction (PCR) from a human IgG1 (pNy1.14) molecule lacking the CH₂ domain. Two oligonucleotides (Sense 49mer, Antisense 50mer) homologous to the sequences of
5 IgG1 constant region at both sides preserving E.co47-III sites were synthesized. The amplified hinge and CH₃ domain PCR fragments were added into Eco47-III sites on BR96 IgG1 molecules by in vivo homologous recombination (P. Bubeck et al., Nucleic Acid Research (1993) 21:3601-3602). The new BR96 IgG1 molecules were verified by restriction mapping and sequencing.

10

A sewing PCR strategy was used for the construction of CH₂ deleted human IgG1 (pNy1.14) (Robert M. Horton, et al. (1990) Biotech 8 (5)P, 528).

The CH₁ domain was amplified as a 580 bp fragment with a sense oligonucleotide
15 (5' TGG CAC CGA AAG CTT TCT GGG GCA GGC CAG GCC TGA 3') (primer A) and an antisense oligonucleotide (5' TCC GAG CAT GTT GGT ACC CAC GTG GTG GTC GAC GCT GAG CCT GGC TTC GAG CAG ACA 3') (primer B) from a linearized human IgG1 constant region vector (pNy1.7). The PCR fragment extends from the 5' end of the Hind-III site (in bold) through the Cel-II, Sal-I, Dra-
20 III, Kpn-I, 6 bp nucleotide spacer and Mro-I sites (in bold) at the 3' end of the CH₁ domain.

The CH₃ domain was then partially amplified (to the Xba-I site) with a sense primer
25 (5' GTC GAC CAC CAC GTG GGT ACC AAC ATG TCC GGA GCC ACA TGG ACA GAG GCC GGC T 3') (primer C) and an antisense primer (5' CTG GTT CTT GTT CAT CTC CTC TCT AGA TGG 3') (primer D) from a linearized human IgG1 constant region vector (pNy1.7). A PCR fragment (about 150 bp) with Sal-I, Dra-III, Kpn-I, 6 nucleotide spacer and Mro-I sites (in bold) on its 5' end, extends only through the Xba-1 site (in bold) within the CH₃ domain.

The CH₁ and CH₃ partial PCR fragments were combined in a PCR without any primer. The reaction was run through two full cycles of denaturation and re-annealing to allow the fragments to combine at the homologous region at the 3' ends. Primers A and D (described above) were added to the reaction and the PCR cycle was completed. The polymerase extends the DNA with primer A and primer D, yielding a full-length (660 bp) PCR fragment. The newly extended PCR fragment is arranged from the 5' end to the 3' end in the following order: Hind-III - CH₁ - Cel-II - Sal-I - Dra-III - Kpn-I - 6 bp spacer - Mro-I - CH₃ partial - Xba-I.

10

The combined PCR fragment, with the CH₁ and partial CH₃ domains, was then cloned by a blunt end ligation into a Sma-I site on a pEMBL18 vector and the sequence was confirmed by dideoxy sequencing (Sanger et al. (1977) PNAS (USA) 74:5463-5466).

15

To transfer the CH₁ and partial CH₃ into a mammalian expression vector, both the pEMBL18 and pNy1.7 vectors were digested with Hind-III and Xba-I. The Hind-III and Xba-I fragment was ligated into the same sites on a linearized pNy1.7 vector. The new construct, with CH₁ and a full CH₃ domain, was designated the pNy1.10 vector.

20

The hinge fragment was amplified from a Hind-III digested pNy1.7 vector with the primers designed to flank the hinge exon with a Sal-I and a Dra-III cloning site at each end. These sites also exist between the CH₁ and CH₃ domains of the pNy1.10 construct. The sense oligonucleotide (5' ACC ATG **GTC GAC CTC AGA CCT** GCC AAG AGC CAT ATC 3') with a 6 bp spacer and a Sal-I cloning site (in bold) and the antisense oligonucleotide (5' CAT GGT CAC **GTG GTG TGT CCC TGG** ATG CAG GCT ACT CTA G 3') with a 6 bp spacer and a Dra-III cloning site (in bold) were used for the amplification of the hinge fragment (250 bp).

25

The hinge region PCR fragment was cloned into a Sma-I site on pEMBL18 by blunt end ligation. Both the pEMBL18 with the hinge domain and the pNy1.10 with the CH₂ and CH₃ domains were digested with Sal-I and Dra-III. The digested hinge
5 fragment was cloned into the Sal-I and Dra-III linearized sites on the pNy1.10 vector. The new construct, now carrying the CH₁, hinge and CH₃ domains, was designated pNy1.11.

To make the final CH₂ deleted human IgG1 construct, both the pNy1.11 construct
10 and pNy1.11 vector were digested with BamHI and HindIII. A fragment containing the CH₁, hinge and CH₃ domains was cloned into the linearized pNy1.11 vector. The new constant region IgG1 construct lacks the CH₂ domain and is designated pNy1.14 (Figure 11).

15 For digestion of BR96 IgG1 with Eco47-III, a restriction fragment with hinge, CH₂ and CH₃ domains was identified on the constant region sequence of BR96 IgG1 vector in both chimeric and humanized molecules. The 5' end of this fragment lies inside the intron between CH₁ and hinge and the 3' end is located inside the CH₃ intron of the BR96 IgG1 molecule. The hinge, CH₂ and CH₃ domains (1.368 kb
20 fragment) were removed from BR96 IgG1 molecules by Eco47-III restriction digestion. The Eco47-III is a blunt end cutter. The BR96 IgG1 DNA digested with this enzyme does not require any pretreatment before cloning. Figure 12 is a diagrammatic representation of the pD17-hBR96-2 vector showing the Eco47-III sites used in cloning.

25

The CH₂ deleted BR96 IgG1 was then constructed as follows. The hinge and CH₃ domains were amplified from a CH₂ deleted L6 IgG1 (pNy1.14) construct with a sense oligonucleotide (5'

CAGGGAGGGAGGGTGTCTGCTGGAAGCCAGGCTCAGCGCTGACCTCAG

- A 3') homologous to the constant region sequence of IgG1 at the 5' end of the Eco47-III site (in bold) and an antisense oligonucleotide
(5'GGAAAGAACCATCACAGTCTCGCAGGGG
CCCAGGGCAGCGCTGGGTGCTT 3') homologous to the constant region
- 5 sequence of IgG1 at the 3' end of the Eco47-III site (in bold). The Eco47-III site at the 3' end of the pN γ 1.14 construct is modified in the cloning process. The Eco47-III site is thus introduced into an antisense primer and used in amplification of the hinge and CH₃ domains.
- 10 The pD17-BR96 IgG1 vector was digested with Eco47-III and the hinge, CH₂ and CH₃ domains were removed. The linearized pD17-BR96 IgG1 vector was mixed with equimolar amounts of hinge and CH₃ PCR fragments. Cotransformation of the PCR fragment with linearized DNA into E.coli DH5a competent cells resulted in a recombinant molecule, mediated by homologous recombination in bacteria. This
- 15 construct lacks the CH₂ domain of BR96 IgG1 molecules, and is designated pD17-BR96-dCH2 (Figure 13).

1.9 grams of CH₂-deleted chimeric BR96 was obtained as raw material from 89L of culture supernatant.

20

EXAMPLE 3

Toxicity, localization and clearance of CH₂-deleted chimeric BR96 was tested in vivo as follows.

25

Three dogs received 400 mg/m² of cBR96-A, the CH₂ deletion mutant of chimeric BR96, and two received chimeric BR96. Both molecules had been mildly reduced and alkylated. This is required to prevent dimerization of the deletion mutant into a tetravalent form. Both control dogs experienced the typical GI toxicity and none of

the three receiving the mutant displayed any toxicity. The control dogs and two of the test dogs were sacrificed at 1 hr to obtain duodenal tissue to measure antibody localization. Both control dogs had grossly visible GI pathology, and the test dogs had normal appearing GI tissue. The third dog has continued to show no signs of

5 toxicity.

Results: A significant amount of localization of the CH₂ deleted cBR96 (cBR96-A) occurred to the GI tract in dogs treated with 400 mg/m², although the intact chiBR96 localized slightly better. The levels of localization indicate that roughly equivalent

10 amounts of intact and CH₂ deleted cBR96 was delivered to the GI tract in these dogs.

Table 5. Localization of cBR96 to GI tissue.

Group	Animal	Specific	mean
Localization			
cBR96	#271	155	135
	#272	114	
cBR96-A	#273	126	89
	#274	52	

15

Using the mean level of specific localization, an amount of cBR96-A equivalent to at least 66% of the amount of cBR96 was delivered to the target organ of toxicity, the duodenum. Based on the dose ranging done with cBR96 in dogs (some clinical

20 signs of toxicity seen at doses of 10 mg/m²), even if this difference is real, it could

not explain the difference between significant toxicity and no toxicity, evaluation to date indicated that dogs treated with cBR96-A had no toxicity, pending microscopic histopathologic examination. This evaluation was based on analysis of 2 frozen blocks per dog and 2 sections per block. Replicates were quite good. We also ran
5 historical frozen tissues from dogs treated with native cBR96 or F(ab)2/BR96 and the levels of localization for those tissues were 110 and 0, respectively, consistent with our previous data.

Assuming that there is no toxicity at marginally higher (2X) doses of cBR96-A,
10 these data indicate that the CH₂ domain is associated with the induction of acute gastroenteropathy, and that the removal of this domain prevents the induction of gastroenteropathy mediated by BR96.

This study confirms the results showing that F(ab')₂ is not toxic in the dog model
15 and that the toxicity is mediated by the constant region. The CH₂ deletion mutant is a candidate for targeting agents clinically. Because of the very long half-life of chimeric BR96, some decrease in the mutant's half-life should be acceptable.

Figure 1 shows the measurement of the clearance of the cBR96-A in high Le^Y
20 expressing dogs. The study used chimeric versus constant region mutant of cBR96-2.

cBR96-2 did clear faster than the chimeric BR96. The localization of cBR96-A to the gastrointestinal epithelium is not significantly affected by this more rapid
25 clearance. More than enough of the cBR96-A localized to have caused toxicity.

Discussion: The constant region of chimeric IgG is responsible for the GI toxicity seen in clinical trials, e.g. with chiBR96-dox. The GI toxicity seen in the dog model is very similar to the clinical toxicity. Both in man and dog, administration of the

unconjugated antibody mediates an acute GI toxicity characterized by rapid onset of vomiting, often with blood.

5 In man the bleeding is limited to the fundus of the stomach, causing erosion of the superficial mucosa of the stomach. Although the pathology of the wound is limited and resolves, the extreme nature of the nausea and vomiting, unrelieved by anti-emetics, defines it as the dose-limiting toxicity.

10 This toxicity is mediated in man and dog by the antibody molecule alone. At higher doses of the antibody-dox conjugate, additional toxicity is seen in the dog model, probably due to doxorubicin. Although the intact IgG of BR96 causes toxicity in dog and man, the F(ab')₂ molecule (divalent and lacking only in the constant region) is not toxic in dogs. This finding has motivated our attempts at high levels, and improves the affinity and specificity of BR96 for tumor antigen.

15

The CH₂ domain is known to mediate complement and FcR binding. It was not known that structural alteration of the CH₂ domain would result in immunoglobulin-induced toxicity inhibition.

20 Toxicology study of hBR96-2B

The toxicology study of hBR96-2B in high Lewis Y expressor dogs (n=2) showed that a dose of 400 mg/m² did not cause hematemesis nor bloody stools, in contrast to BR96 which consistently causes one or both signs. A dog sacrificed at 24 hrs had
25 normal gross appearance of the GI tract, again in marked contrast to chimeric BR96 which causes hemorrhagic lesions and mucosal erosions.

EXAMPLE 4

- The polymerase chain reaction (PCR) is a widely used and versatile technique for the amplification and subsequent modification of immunoglobulin genes. The
- 5 rapidity and accuracy with which antibody genes can be modified in vitro has produced an assortment of novel antibody genes can be modified in vitro has produced an assortment of novel antibodies. For example, PCR methods have been used for engineering antibodies with increased affinity to antigen, for "humanizing" antibodies, and for modulating effector function (Marks, J.D., A.D. Griffiths, M.
- 10 Malmqvist, T. Clackson, J.M. Bye and G. Winter. 1992. Bypassing immunization: high affinity human antibodies by chain shuffling. *Bio/Technology* 10:779-783; Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96
- 15 Fab. *J. Biol. Chem.* 271:22611-22618; Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology*. 86:319-324).
- 20 As part of a more comprehensive study, we desired to introduce various site specific mutations in the CH₂ constant domain of human IgG₁. Six specific amino acid residues distributed throughout the CH2 domain previously identified to play a role in immune effector function were marked as targets for mutagenesis (Morgan, A.N., D. Jones, A.M. Nesbitt, L. Chaplin, M.W. Bodmer and S. Emtage. 1995. The N-
- 25 terminal end of the CH2 domain of chimeric human IgG1 anti-HLA-DR is necessary for Clq, FcγRI and FcγRIII binding. *Immunology*. 86:319-324; Duncan, A.R. and G. Winter. 1988. The binding site for C1q on IgG. *Nature* 332:738-740; Tao, M.-H., R.I.F. Smith and S.L. Morrison. 1993. Structural features of human immunoglobulin G that determine isotype-specific differences in complement

activation. J.Exp.Med. 178:661-667). five of the six residues were grouped into two clusters-one cluster consisting of two residues, two amino acids apart (Location 1, or L1); and a second cluster consisting of three residues spanning a sequence of five amino acids (L2). The remaining amino acid position (L3) made for the total of six
5 residues. We were interested in constructing a panel of mutant CH₂ domain IgGs consisting of each L mutation by itself as well as in combination with other L mutants (e.g., L1; L1; and L2; L1, L2 and L3; etc.).

Various *in vitro* methods have been described where PCR is used to simultaneously
10 introduce distally located site-specific mutations within a gene sequence (Ho, S.N., H.D. Hunt, R.M. Horton, J.K. Pullen and L.R. Pease. 1989. Site-directed mutagenesis by overlap extension. Gene 77:51-59; Ge, L. and P. Rudolph. 1996. Simultaneous introduction of multiple mutations using overlap extension PCR. BioTechniques 22:28-30). Alternatively, an *in vivo* procedure termed recombination
15 PCR (RPCR) has also successfully been used for rapidly and efficiently generating distally located site-specific mutations (Jones, D.H. and S.C. Winistorfer. 1993. Use of polymerase chain reaction for making recombinant constructs. p.241-250. In B.A. White (Ed.), Methods in Molecular Biology, Vol. 15. Humana Press Inc., Totowa, NJ, Jones, D.H. And B.H. Howard. 1991. A rapid method for
20 recombination and site-specific mutagenesis by placing homologous ends on DNA using polymerase chain reaction. BioTechniques 10:62-66). RPCR uses *E. Coli*'s recombination machinery to generate intact circular recombinant plasmids from a transfected mixture of linear PCR-generated product and linearized vector. *In vivo* recombination is mediated through the joining of nucleotide sequences designed into
25 the 5' ends of both PCR primers that are homologous to DNA sequences encoded by the vector. In this report we describe an extension of the RPCR procedure for simultaneously introducing complex combinations of mutations into an antibody CH₂ domain.

Humanized BR96 variable region heavy and light chain genes, previously cloned and co-expressed as an assembled active Fab fragment in an M13 phage expression vector, provided the starting material (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K.-E. Hellstrom, I. Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D.

- 5 Huse and S.M. Glaser. 1996. A combinatorial library strategy for the rapid humanization of anticarcinoma BR96 Fab. *J. Biol. Chem.* 271:22611-22618). The heavy and light chain V genes were amplified by PCR from a single-stranded M13 DNA template and subcloned by *in vivo* recombination (Jones, D.H. And B.H. Howard. 1991. A rapid method for recombination and site-specific mutagenesis by
10 placing homologous ends on DNA using polymerase chain reaction. *BioTechniques* 10:62-66) into vectors pD17-hG1a and pD16-hC κ , to form pBR96-hG1a and pBR96-hC κ respectively. pD17-hG1a and pD16-hC κ are eukaryotic immunoglobulin expression vectors derived from pcDNA3 (Invitrogen, San Diego, CA). The plasmid pBR96-hG1a was further modified by site-directed mutagenesis
15 to introduce two Eco47-III restriction sites flanking the immunoglobulin hinge-CH₂-CH₃ domains using standard procedures. The recipient vector was then prepared by digesting pBR96-hG1a with Eco47-III, isolating the vector backbone by agarose gel electrophoresis followed by extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA).

20

- The strategy for introducing multiple mutations within the immunoglobulin CH₂ gene, shown in Figure 24, relies on the *in vivo* homologous recombination of several independently amplified PCR products with each other as well as with the pBR96-hG1a vector DNA. For introducing mutations at two distal locations two PCR
25 products are synthesized (Figure 24B). One end of each PCR product is for recombining with an homologous end of the linear vector, and the other end, encoding the mutation(s) of interest, is for recombining with the neighboring PCR product. As shown in Figure 24B, additional distally-located mutations can be introduced into a target sequence by increasing the number of PCR products

proportionately. The recombination of neighboring PCR products always occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends (e.g., A1, A2) contain complementary mutant residues.

The mutagenic PCR primers contain at least 15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers (Rs and Ra) contain sequences for recombining with the end regions of the Eco47-III digested pBR96-hG1a vector.

Each L mutation was amplified in a separate PCR reaction. The reaction conditions were 250 ng intact pBR96-hG1a DNA template, 10 ul of 1X *Pfu* buffer (Stratagene, Inc. San Diego, CA), 10 nmol dNTPs, 200ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase in a 100ul reaction volume. Samples were first denatured at 95° C for 5 min, cooled to 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, followed by a final extension at 72°C for 7 min in a Perkin-Elmer DNA Thermal Cycler (Norwalk, CT). The amplified products were purified from a 1% agarose gel, extracted with Qiagen Gel Extraction kit and the recovered DNA quantitated. 50 ng of each PCR product was mixed with 25 ng of the Eco47-III digested pBR96-hG1a vector, transfected into Max competent *E. coli* DH5α according to the manufacturer's procedure (GIBCO BRL/Life Technologies, Gaithersburg, MD), and the entire transfection reaction plated onto selective LB agar plates containing 100 ug/ml ampicillin.

The results of several cloning experiments are summarized in the Table that follows. Typically the transformations produced from 80 to 200 bacterial colonies. Individual colonies were selected and grown overnight in 2 ml liquid cultures for isolation of miniprep plasmid DNA (Qiagen) and analysis by Eco47-III restriction

endonuclease mapping. Among 24 independent transformants analyzed from triple homologous recombination events (two PCR products plus vector) 11 clones contained the predicted 1.4 kpb DNA insert.

- 5 Figure 25 shows a sample diagnostic restriction analysis of DNA prepared from clones derived from quadruple homologous recombination events (three PCR products plus vector). Additional sampling of clones resulting from quadruple recombination yielded a cloning efficiency of 29% (7 clones containing inserts/24 clones sampled). At this point, due to the small sampling sizes, we do not know
10 whether the differences in the cloning efficiencies observed between the triple and quadruple recombination events are meaningful.

- To evaluate the expression of Le γ -binding activity of the CH₂ mutant IgGs, miniprep DNAs from 6 clones derived from the triple recombination reaction and 6
15 clones derived from the quadruple recombination reaction exhibiting the predicted diagnostic Eco47-III restriction patterns were isolated, mixed with pBR96- hCk DNA and used to co-transfect COS7 cells. 48 hour spent supernatants from 3 ml cultures were assayed for total IgG production and for Le γ binding activity by enzyme-linked immunosorbent assay (EIA) as described (Yelton, D.E., M.J. Rosok,
20 G.A. Cruz, W.L. Cosand, J. Bajorath, I. Hellstom, K.-E. Hellstorm, W.D. Huse and S.M. Glaser. 1995. Affinity maturation of the BR96 anti-carcinoma antibody by codon-based mutagenesis. J.Immunol. 155:1994-2004). All twelve cultures were found to secrete approximately 2-3 ug/ml Le γ -reactive IgG. The spectrum of Le γ binding activities were all similar to that of native humanized BR96 IgG indicating
25 that the homologously recombined antibodies did not acquire any gross mutations that could affect antigen binding. To confirm that the desired CH₂ mutations had been incorporated, and to evaluate the recombined genes for misincorporated nucleotides, four of the clones producing functional antibody were sequenced using Sequenase Version 2 DNA Sequencing Kit (United States Biochemical). One clone

was found to contain a single nucleotide change within the forward PCR primer used for mediating recombination with vector DNA. We are uncertain whether this error occurred during chemical synthesis of the oligonucleotide primer or is a result of misincorporation during the PCR reaction, despite the fact that we used a

5 thermostable polymerase with proofreading activity.

A RPCR procedure for homologously recombining up to three separate PCR-generated mutated antibody sequence products into a eukaryotic expression vector for the rapid construction of engineered IgG molecules is described herein. The

10 advantage of this approach is the ability to simultaneously introduce multiple distally-located mutations with PCR products synthesized by a single round of PCR. Recombinant DNAs are produced with a reasonably high cloning efficiency and fidelity of correct nucleotide sequences. The ability to efficiently rejoin several

15 distinct PCR products should permit combinatorial strategies for constructing complexly mutated protein domains as well as broadening the number and location of desired mutations.

Analysis of transformants generated by multiple-fragment RPCR.

Mutant IgGs Constructed	PCR Fragments in reaction	HR ^a events	Colonies Analyzed	Cloning Efficiency ^b
2	2	triple	24	45%
2	3	quadruple	24	33%
^a HR-homologous recombination				
^b Cloning efficiency (number of clones containing 1.4kbp insert/total number of colonies)				

EXAMPLE 5

This example provides two methods for introducing site specific mutations into the
5 CH2 domain of human IgG1 constant region containing vectors.

One method involves PCR amplification of a segment or segments of the constant region, wherein mutations are introduced using appropriately constructed oligonucleotides. The vector receiving the fragment(s) is digested with a restriction
10 enzyme to linearize the vector. PCR amplification primers are designed so that the 5' ends of the PCR fragments can hybridize to the DNA sequence of the vectors. If more than one PCR fragment is amplified, then common sequences to the two fragments are introduced by oligonucleotides. Bacteria are transfected with the PCR fragments and with the digested vector. The fragments and vector can recombine by
15 homologous recombination using the bacteria's recombination machinery. Bacterial colonies are selected and the DNA is analyzed by size and restriction map as a preliminary determination that the vector and fragment(s) recombined correctly. Correct insertion of fragments with the mutations is confirmed by dideoxynucleotide sequence analysis. DNA is then introduced into mammalian cells as described for
20 the CH2 deleted antibody, and the expressed antibody analyzed for binding and functional activity.

By way of example, mutations Leu to Ala at residue 235 in CH2 and Gly to Ala at residue 237 were introduced by the procedure disclosed in Example 4. The heavy
25 chain vector used for this procedure was pD17-hG1a, similar to pD17-BR96 vector described herein except that humanized V regions (Rosok, M.J., D.E. Yelton, L.J. Harris, J. Bajorath, K-E. Hellstrom, I, Hellstrom, G.A. Cruz, K. Kristensson, H. Lin, W.D. Huse, and S.M. Glaser, 1996. J. Biol. Chem 271 37:22611-22618) with three affinity mutations (H1, H2, and H3 mutations) were substituted.

pBR96-hG1a contains two *Eco47-III* restriction sites flanking the Ig hinge-CH2-CH3 domains. The recipient vector was prepared by (1) digesting pBR96-hG1a with *Eco47-III*, (2) isolating the vector by agarose gel electrophoresis, and (3)
5 extracting the vector DNA from the excised gel slice using the Qiagen Gel Extraction kit (Qiagen, Chatsworth, CA). To introduce mutations at a single location, such as for positions 235 and 237, two PCR products were synthesized.

To introduce two distally located mutations, such as for mutant F (also referred to
10 herein as hBR96-2F) with mutations at 235, 237, 331, requires 3 PCR products. The recombination of neighboring PCR products occurs across the regions containing the desired mutations, therefore the oligonucleotide primers encoding these ends contain complementary mutant residues. The mutagenic PCR primers contain at least 15
15 nucleotides of wild-type sequence flanking each side of the mutant residues for either priming the polymerization reaction or mediating recombination. Two 49-nucleotide long PCR sense and anti-sense primers containing sequences for recombining with the end regions of the *Eco47-III* digested pBR96-hG1a vector.

PCR amplification used 250 ng intact pBR96-hG1a DNA template, 10 μ l of 10X *Pfu*
20 buffer (Stratagene, Inc., San Diego, CA), 10 nmol dNTPs, 200 ng each of the appropriate PCR primers, 10% dimethylsulfoxide (ATCC, Rockville, MD) and 2.5 units cloned *Pfu* DNA polymerase (Stratagen, Inc. San Diego, CA) in 100 μ l reaction. Samples were denatured at 95°C for 5 min, annealed at 45°C for 5 min, and extended at 72°C for 1 min followed by 25 cycles of denaturation at 94°C for 45
25 sec, annealing at 45°C for 45 sec, extension at 72°C for 1 min/kb, and a final extension at 72°C for 7 min. The amplified products were purified from a 1% agarose gel, extracted with the Qiagen Gel Extraction kit and quantitated. 50 mg of each PCR product was mixed with 25 ng of the *Eco47-III* digested pBR96-hG1a vector and transfected in *E. coli* MAX Efficiency DH5 α TM according to the

manufacturer's instructions (GIBCO BRL/Life Technologies, Gaithersburg, MD).

The entire transfection reaction was plated onto LB agar plated containing 100 µg/ml ampicillin.

- 5 Bacterial colonies were selected and grown overnight at 37° C in 2 ml liquid cultures. DNA was isolated and analyzed by Eco47-III restriction endonuclease mapping. Clones with the correct size insert were sequenced (Sequenase Version 2, U.S. Biochemical Corp., Cleveland, OH).
- 10 The second method for introducing site specific mutations into the CH₂ domain of human IgG1 involved the method of Kunkel (1987 Methods Enzymology, supra). For this procedure pD17-hG1b DNA with the F1 origin of replication was introduced into electrocompetent E. coli CJ236 dut-ung- (Bio-Rad Laboratories, Hercules, CA) by electroporation according to manufacturer's instructions. PD17-
15 hG1b is a vector having a constant region but no variable region. The F1 ori site allows treatment of this vector as a phagemid.

Bacteria containing the plasmid were selected by ampicillin resistance. Single stranded uridynylated DNA was prepared using the Muta-Gene Phagemid In Vitro

- 20 Mutagenesis Version 2 protocol (Bio-Rad). Mutations were introduced by site-directed mutagenesis with the appropriate antisense oligonucleotide. For molecules with mutations at more than one location, mutations were introduced by either of the two methods discussed above. One method would be to (1) prepare one mutant, for example, mutant 2C (also referred to herein as BR96-2C) with the mutations at
25 residues 318, 320, 322, (2) isolate ssDNA, and (3) introduce a second mutation set with the appropriate anti-sense oligonucleotide. The second method would be to anneal two antisense oligonucleotides with the same uridynylated ssDNA and screen for mutants with both sets of changes. Mutant 2H (hBR96-2H) was also prepared by a combination of these methods.

The V region of humanized BR96-2 heavy chain was introduced by the homologous recombination method described above in pD17-hJm14.H1. The pD17-hJm14.H1 plasmid contains the BR96 humanized variable region with the H1/H2/H3 mutations and the plasmid was used to transfect mutant sequences into mammalian cells. The pD17G1b vector containing the Fc mutation(s) was digested with NheI for 3 hr at 37° C and the DNA isolated by methods described above. Insertion of the V region into the vector was determined by size and restriction enzyme mapping and confirmed by sequence analysis.

10

Transient expression of whole antibodies was performed by transfection of COS cells. For production of antibody, stable transfections of CHO cells were performed (see description of deleted CH2 mutant). All mutants were purified from CHO culture supernatants by protein A chromatography.

15

The oligonucleotide primers homologous to the vector and used to introduce the constant regions mutations were as follows:

Oligonucleotides homologous to vector sequences:

Sens(sense)CH2 E47-3-5: CAG GGA GGG AGG GTG TCT GCT GGA AGC
20 CAG GCT CAG CGC TGA CCT CAGA
D CH2 E47-3 A (antisense): GGA AAG AAC CAT CAC AGT CTC GCA GGG
GCC CAG GGC AGC GCT GGG TGC TT

Oligonucleotides to mutate Leu235 to Ala and Gly237 to Ala (underlined sequences show sites of mutation):

25 Antisense CH2 L235-G237/aa: GAA GAG GAA GAC TGA CGG TGC CCC
CGC GAG TTC AGG TGC TGA GG
SensCH2 L235-G237/AA: CCT CAG CAC CTG AAC TCG CGG GGG CAC
CGT CAG TCT TCC TCT TC

Oligonucleotides to mutate Glu318, Lys320, Lys322 to Ser

Antis(antisense)CH2 EKK/SSS-2: CTG GGA GGG CTT TGT TGG AGA CCG
AGC ACG AGT ACG ACT TGC CAT TCA GCC

5 Oligonucleotides to mutate Pro331 to Ala:

Antis CH2 P331/A/3: GAT GGT TTT CTC GAT GGC GGC TGG GAG GGC

Sense CH2 P33/A: GCC CTC CCA GCC GCC ATC GAG AAA ACC ATC

Alternative antisense oligo to introduce Ala at 331 by site-directed mutation:

CH2P331A: GAT GGT TTT CTC GAT AGC GGC TGG GAG GGC TTT G

10

Oligonucleotides to mutate Glu318 to Ser, Lys320 to Ser, Lys322 to Ser, and Pro331 to Ala:

Antis CH2 EKKP/SSA-6: GAT GGT TTT CTC GAT GGC GGC TGG GAG
 GGC TTT GTT GGA GAC CGA GCA CGA GTA CGA CTT GCC ATT CAG

15 CCA GTC CTG GTG

Sense CH2 EKKP/SSA-6: CAC CAG GAC TGG CTG AAT GGC AAG TCG
 TAC TCG TGC TCG GTC TCC AAC AAA GCC CTC CCA GCC GCC ATC
 GAG AAA ACC ATC

20

In vitro Assays of the Mutants

Results of the CDC demonstrate that mutant hBR96-2B has approximately 10 fold less activity than the control hBR96-1 (two affinity mutations, one in H2 and one in H3, refer to previous patent (Figure 20)). The mutants that have the least ability to kill cells in the presence of complement is hBR96-2C with the triple mutations at positions 318, 320, and 322 and the hBR96-2H mutant (least cytotoxic antibodies in the panel) which contains all six mutations at the three different locations. ADCC activity was most affected by the CH2 deleted hBR96-2 molecule (Figure 21).

25

hBR96-2B and -2H lost between 100 and 1000 fold activity to kill in the presence of effector cells. In the ADCC assay the hBR96-2B molecule also lost approximately 10 fold activity (Figure 21).

- 5 Figures 26-28 provide the amino acid sequences for the heavy chain variable region for both chimeric and humanized BR96 having the H1, H2, and H3 mutations. The amino acid sequence for the light chain variable region is known and methods for generating it are found in PCT Application No. 95/305444. Additionally provided is the amino acid sequence for the IgG1 constant region. Mutations in the constant
- 10 region are marked.

SEQUENCE LISTING

(1) GENERAL INFORMATION

5

(i) APPLICANT: Bristol-Myers Squibb Co.

(ii) TITLE OF THE INVENTION:

10

A METHOD FOR INHIBITING
IMMUNOGLOBULIN-INDUCED TOXICITY FROM THE USE OF
IMMUNOGLOBULINS IN THERAPY AND IN VIVO DIAGNOSIS

(iii) NUMBER OF SEQUENCES: 13

15

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Merchant & Gould
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(C) CITY: Los Angeles
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 90025

20

(v) COMPUTER READABLE FORM:

25

(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 2.0

30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US97/_____
(B) FILING DATE: 01-AUG-1997
(C) CLASSIFICATION:

35

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/023,033
(B) FILING DATE: 02-AUG-1996

40

(viii) ATTORNEY/AGENT INFORMATION:

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45

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(C) TELEX:

50

(2) INFORMATION FOR SEQ ID NO:1:

55

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 TGGCACCGAA AGCTTTCTGG GGCAGGCCAG GCCTGA 36

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 57 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCCGGACATG TTGGTACCCA CGTGGTGGTC GACGCTGAGC CTGGCTTCGA GCAGACA 57

20 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 55 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTCCGACCACC ACGTGGGTAC CAACATGTCC GGAGCCACAT GGACAGAGGC CGGCT 55

35 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTGGTTCTTG TTCATCTCCT CTCTAGATGG 30

(2) INFORMATION FOR SEQ ID NO:5:

50 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

55 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ACCATGGTCG ACCTCAGACC TGCCAAGAGC CATATC

36

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATGGTCACG TGGTGTGTCC CTGGATGCAG GCTACTCTA

39

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 49 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGGGAGGGA GGGTGTCTGC TGGAAGCCAG GCTCAGCGCT GACCTCAGA

49

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 50 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGAAAGAACC ATCACAGTCT CGCAGGGGCC CAGGGCAGCG CTGGGTGCTT

50

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8691 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACGGATCGG GAGATCTGCT AGGTGACCTG AGGCGCGCCG GCTTCGAATA GCCAGAGTAA
CCTTTT TTTT TAATTTTATT TTAATTTTATT TTTGAGATGG AGTTTGCGC CGATCTCCCG

60

120

	ATCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTGCGTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
5	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
10	GGGACTTTCC	TACTTGGCAG	TACATCTACG	TATTAGTCAT	CGCTATTACC	ATGGTGATGC	720
	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AAATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
15	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGCGGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCTT	TCCTTGTCCT	TGTTTTAAAA	GGTGTCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
20	TCTCCTGTGT	AACCTCTGGA	TTCACTTTCA	GTGACTATTA	CATGTATTGG	GTTGCCCAGA	1260
	CTCCAGAGAA	GAGGCTGGAG	TGGGTGCGAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTACCA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAAT	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCACG	GTCTCTGTAG	1500
25	CTAGACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCGT	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCTCTAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCAACG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTTGGTGAGA	1800
30	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCCCTC	TGCCCGCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCTT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTTGA	CCTAAGCCCA	2100
35	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAATCTCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACAGGCCCCA	GCCGGGTGCT	GACACGTCCA	CCTCCATCTC	2340
	TTCTCTCAGCA	CCTGAACTCC	TGGGGGGACC	GTCACTCTTC	CTCTTCCCCC	CAAAACCCAA	2400
40	GGACACCCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGTGGTGG	ACGTGAGCCA	2460
	CGAAGACCCT	GAGGTCAAGT	TCAACTGGTA	CGTGGACGGC	GTGGAGGTGC	ATAATGCCAA	2520
	GACAAAGCCG	CGGGAGGAGC	AGTACAACAG	CACGTACCGT	GTGGTCAGCG	TCCTCACCGT	2580
	CCTGCACCAG	GACTGGCTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	2640
	CCCAGCCCCC	ATCGAGAAAA	CCATCTCCAA	AGCCAAAGGT	GGGACCCGTG	GGGTGCGAGG	2700
45	GCCACATGGA	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	2760
	CCTCTGTCCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	2820
	ATGAGCTGAC	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCCAGCG	2880
	ACATCGCGGT	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCCT	2940
	CCGTGCTGGA	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCCTG	GACAAGAGCA	3000
50	GGTGCGAGCA	GGGGAACGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACCACT	3060
	ACACGCAGAA	GAGCCTCTCC	CTGTCTCCGG	GTAATAGAGT	GCGACGGCCG	GCAAGCCCCC	3120
	GCTCCCCGGG	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTTG	TACATACTTC	3180
	CCGGGCGCCC	AGCATGGA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCTTG	CGAGACTGTG	3240
	ATGGTTCTTT	CCACGGGTCA	GGCCGAGTCT	GAGGCCTGAG	TGGCATGAGG	GAGGCAGAGC	3300
55	GGGTCCCACT	GTCCCCACAC	TGGCCACAGC	TGTGCAGGTG	TGCCTGGGCC	CCCTAGGGTG	3360
	GGGCTCAGCC	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	3420
	AGCAGCACCT	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTGGG	GACAGACACA	3480
	CAGCCCTGTC	CTCTGTAGGA	GACTGTCTTG	TTCTGTGAGC	GCCCCTGTCC	TCCCGACCTC	3540
	CATGCCCACT	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	3600
	CTACCCCCAC	GGCACTAACC	CCTGGCTGCC	CTGCCAGGCC	TCGCACCCGC	ATGGGGACAC	3660

	AACCGACTCC	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GA CTGGTGCA	GATGCCACCA	3720
	CACACACTCA	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	3780
	CACCACACAC	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	3840
	CCCAGACCAG	AGCAAGGTCC	TGCGACACGT	GAACACTCCT	CGGACACAGG	CCCCACGAG	3900
5	CCCCACGCGG	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	3960
	TCAGACAAAC	CCAGCCCTCC	TCTCACAAGG	GTGCCCTTGC	AGCGCCACCA	CACACACAGG	4020
	GGATCACACA	CCACGTACAG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	4080
	CAGGACGGAT	CAGCCTCGAC	TGTGCCCTTCT	AGTTGCCAGC	CATCTGTTGT	TTGCCCCCTCC	4140
	CCCGTGCCCT	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	4200
10	GAAATTGCAT	CGCATTGTCT	GAGTAGGTGT	CATTCTATTG	TGGGGGGTGG	GGTGGGGCAG	4260
	GACAGCAAGG	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	4320
	ATGGCTTCTG	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	4380
	AGCGGCGCAT	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCCG	TACACTTGCC	4440
	AGCGCCCTAG	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTGCGCGGG	4500
15	CCTCTCAAAA	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	4560
	CTAACTCCGC	CCATCCCGCC	CCTAACTCCG	CCCAGTTCGG	CCCATTCTCC	GCCCCATGGC	4620
	TGACTAATTT	TTTTTATTTA	TGCAGAGGCC	GAGGCCGCTC	CGGCCTCTGA	GCTATTCCAG	4680
	AAGTAGTGAG	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	4740
	GCTGCGATTG	CGCGCCAAAC	TGACGCGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	4800
20	CCCGCTGCCA	TCATGGTTCG	ACCATTGAAC	TGCATCGTCG	CCGTGTCCCA	AAATATGGGG	4860
	ATTGGCAAGA	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTACTTCCAA	4920
	AGAATGACCA	CAACCTCTTC	AGTGGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	4980
	ACCTGGTTCT	CCATTCTCGA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	5040
	AGTAGGAAC	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCAAAAAG	TTTGGATGAT	5100
25	GCCTTAAGAC	TTATTGAACA	ACCGGAATG	GCAAGTAAAG	TAGACATGGT	TTGGATAGTC	5160
	GGAGGCAGTT	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	5220
	ACAAGGATCA	TGCAGGAATT	TGAAAGTGAC	ACGTTTTCCT	CAGAAATTGA	TTTGGGGAAA	5280
	TATAAACTTC	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	5340
	AAGTATAAGT	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	5400
30	GCTCCCCTCC	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	5460
	TCTTTGTGAA	GGAACTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	5520
	TTTAAAGCTC	TAAGGTAAAT	ATAAAATTTT	TAAGTGATA	ATGTGTTAAA	CTACTGATTG	5580
	TAATGTGTTG	TGTATTTTAG	ATTCCAACCT	ATGGAAGTGA	TGAATGGGAG	CAGTGGTGGA	5640
	ATGCTTTTAA	TGAGGAAAC	CTGTTTGTCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	5700
35	CTACTGCTGA	CTCTCAACAT	TCTACTCTCT	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	5760
	AGGACTTTCC	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	5820
	TTGCTTGCTT	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	5880
	TGGAAAATA	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	5940
	TTTTTCTTAC	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTTG	6000
40	GTACCTTTAG	CTTTTAAATT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCCT	6060
	TGACTAGAGA	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	6120
	CTCCACACAC	TCCCCTGAA	CCTGAAACAT	AAATGAATG	CAATTGTTGT	TGTTAACTTG	6180
	TTTATTGCAG	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	6240
	GCATTTTTTT	CACGCAATTC	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	6300
45	GTCTGGATCG	GCTGGATGAT	CCTCCAGCGC	GGGATCTCA	TGCTGGAGTT	CTTCGCCCAC	6360
	CCCAACTTGT	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	6420
	ACAAATAAAG	CATTTTTTTC	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	6480
	TCTTATCATG	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	6540
	CTGTTTCTCT	TGTGAAATTG	TTATCGGCTC	ACAATTCCAC	ACAACATACG	AGCCGGAAGC	6600
50	ATAAAGTGTA	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	6660
	TCACTGCCCC	CTTTCCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	6720
	CGCGCGGGGA	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCGG	CTTCCTCGCT	CACTGACTCG	6780
	CTGCGCTCGG	TCGTTCCGGCT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	6840
	TTATCCACAG	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	6900
55	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	6960
	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	7020
	TACCGAGCGT	TTCCCCTTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCGGCTT	7080
	ACCGGATACC	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	7140
	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	7200

	CCCCGTTGAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	7260
	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	7320
	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	7380
	GTATTGGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	7440
5	TGATCCGGCA	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	7500
	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	7560
	CAGTGGAAAC	AAAACCTACG	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	7620
	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	7680
	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	7740
10	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTGCTGTAGA	TAACTACGAT	ACGGGAGGGC	7800
	TTACCATCTG	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	7860
	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	7920
	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCCAGTT	7980
	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	8040
15	GGTAGGGCTT	CATTGAGCTC	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	8100
	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	8160
	GCAGTGTTAT	CATCATGGT	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	8220
	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	8280
	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	8340
20	ACTTTAAAAG	TGCTCATCAT	TGGAAAACGT	TCITCGGGGC	GAAAACTCTC	AAGGATCTTA	8400
	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACGATC	TTCAGCATCT	8460
	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	8520
	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTC	ATATTATTGA	8580
	AGCATTATAT	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	8640
25	AAACAAATAG	GGGTTCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	C	8691

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 8327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

	GACGGATCGG	GAGATCTGCT	AGGTGACCTG	AGGCGCGCGG	GCTTCGAATA	GCCAGAGTAA	60
40	CCTTTTTTTT	TAATTTTATT	TTATTTTATT	TTTGAGATGG	AGTTTGGCGC	CGATCTCCCG	120
	ATCCCCCTATG	GTCGACTCTC	AGTACAATCT	GCTCTGATGC	CGCATAGTTA	AGCCAGTATC	180
	TGCTCCCTGC	TTGTGTGTTG	GAGGTGCTG	AGTAGTGCGC	GAGCAAAATT	TAAGCTACAA	240
	CAAGGCAAGG	CTTGACCGAC	AATTGCATGA	AGAATCTGCT	TAGGGTTAGG	CGTTTTGCGC	300
	TGCTTCGCGA	TGTACGGGCC	AGATATACGC	GTTGACATTG	ATTATTGACT	AGTTATTAAT	360
45	AGTAATCAAT	TACGGGGTCA	TTAGTTCATA	GCCCATATAT	GGAGTTCCGC	GTTACATAAC	420
	TTACGGTAAA	TGGCCCGCCT	GGCTGACCGC	CCAACGACCC	CCGCCCATTG	ACGTCAATAA	480
	TGACGTATGT	TCCCATAGTA	ACGCCAATAG	GGACTTTCCA	TTGACGTCAA	TGGGTGGACT	540
	ATTTACGGTA	AACTGCCAC	TTGGCAGTAC	ATCAAGTGTA	TCATATGCCA	AGTACGCCCC	600
	CTATTGACGT	CAATGACGGT	AAATGGCCCC	CCTGGCATT	TGCCCAGTAC	ATGACCTTAT	660
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	GGTTTTGGCA	GTACATCAAT	GGGCGTGGAT	AGCGGTTTGA	CTCACGGGGA	TTTCCAAGTC	780
	TCCACCCCAT	TGACGTCAAT	GGGAGTTTGT	TTTGGCACCA	AAATCAACGG	GACTTTCCAA	840
	AATGTCGTAA	CAACTCCGCC	CCATTGACGC	AAATGGGCGG	TAGGCGTGTA	CGGTGGGAGG	900
	TCTATATAAG	CAGAGCTCTC	TGGCTAACTA	GAGAACCCAC	TGCTTACTGG	CTTATCGAAA	960
55	TTAATACGAC	TCACTATAGG	GAGACCCAAG	CTTGGTACCA	ATTTAAATTG	ATATCTCCTT	1020
	AGGTCTCGAG	TCTCTAGATA	ACCGGTCAAT	CGATTGGAAT	TCTTGGCGCC	GCTTGCTAGC	1080
	CACCATGGAG	TTGTGGTTAA	GCTTGGTCCT	TCCTTGTCTT	TGTTTTAAAA	GGTGTCAGT	1140
	GTGAAGTGAA	TCTGGTGGAG	TCTGGGGGAG	GCTTAGTGCA	GCCTGGAGGG	TCCCTGAAAG	1200
	TCTCTGTGT	AACCTCTGGA	TTCATTTTCA	GTGACTATTA	CATGTATTGG	GTTCCGCCAGA	1260

	CTCCAGAGAA	GAGGCTGGAG	TGGGTCGCAT	ACATTAGTCA	AGGTGGTGAT	ATAACCGACT	1320
	ATCCAGACAC	TGTAAAGGGT	CGATTCAACA	TCTCCAGAGA	CAATGCCAAG	AACACCCTGT	1380
	ACCTGCAAA	GAGCCGTCTG	AAGTCTGAGG	ACACAGCCAT	GTATTACTGT	GCAAGAGGCC	1440
	TGGACGACGG	GGCCTGGTTT	GCTTACTGGG	GCCAAGGGAC	TCTGGTCAGG	GTCTCTGTAG	1500
5	CTAGCACCAA	GGGCCCATCG	GTCTTCCCCC	TGGCACCCCTC	CTCCAAGAGC	ACCTCTGGGG	1560
	GCACAGCGGC	CCTGGGCTGC	CTGGTCAAGG	ACTACTTCCC	CGAACCGGTG	ACGGTGTCTG	1620
	GGAACTCAGG	CGCCCTGACC	AGCGGCGTGC	ACACCTTCCC	GGCTGTCTTA	CAGTCCTCAG	1680
	GACTCTACTC	CCTCAGCAGC	GTGGTCACCG	TGCCCTCCAG	CAGCTTGGGC	ACCCAGACCT	1740
	ACATCTGCAA	CGTGAATCAC	AAGCCCAGCA	ACACCAAGGT	GGACAAGAAA	GTGGTGTAGA	1800
10	GGCCAGCACA	GGGAGGGAGG	GTGTCTGCTG	GAAGCCAGGC	TCAGCGCTCC	TGCCTGGACG	1860
	CATCCCGGCT	ATGCAGCCCC	AGTCCAGGGC	AGCAAGGCAG	GCCCCGTCTG	CCTCTTCACC	1920
	CGGAGGCTC	TGCCCCCCCC	ACTCATGCTC	AGGGAGAGGG	TCTTCTGGCT	TTTTCCTCCAG	1980
	GCTCTGGGCA	GGCACAGGCT	AGGTGCCCCCT	AACCCAGGCC	CTGCACACAA	AGGGGCAGGT	2040
	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC	CGGGAGGACC	CTGCCCTTGA	CCTAAGCCCA	2100
15	CCCCAAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA	2160
	GTAATCCCA	ATCTTCTCTC	TGCAGAGCCC	AAATCTTGTG	ACAAAACCTCA	CACATGCCCA	2220
	CCGTGCCCAG	GTAAGCCAGC	CCAGGCCTCG	CCCTCCAGCT	CAAGGCGGGA	CAGGTGCCCT	2280
	AGAGTAGCCT	GCATCCAGGG	ACACACCACG	TGGGTACCAA	CATGTCCGGA	GCCACATGGA	2340
	CAGAGGCCGG	CTCGGCCAC	CCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCTCTGTCCC	2400
20	TACAGGCGAG	CCCCGAGAAC	CACAGGTGTA	CACCCTGCCC	CCATCCCGGG	ATGAGCTGAC	2460
	CAAGAACCAG	GTCAGCCTGA	CCTGCCTGGT	CAAAGGCTTC	TATCCAGCG	ACATCGCGT	2520
	GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAAG	ACCACGCCCTC	CCGTGCTGGA	2580
	CTCCGACGGC	TCCTTCTTCC	TCTACAGCAA	GCTCACCGTG	GACAAGAGCA	GGTGGCAGCA	2640
	GGGGAACGTC	TTCTCATGCT	CCGTGATGTC	TGAGGCTCTG	CACAACCACT	ACACGCAGAA	2700
25	GAGCCTCTCC	CTGTCTCCGG	GTAATGAGT	GCGACGGCCG	GCAAGCCCCC	GCTCCCCGGG	2760
	CTCTCGCGGT	CGCACGAGGA	TGCTTGGCAC	GTACCCCTG	TACATACTTC	CCGGGCGCCC	2820
	AGCATGAAAA	TAAAGCACCC	AGCGCTGCCC	TGGGCCCTG	CGAGACTGTG	ATGTTCTTT	2880
	CCACGGGTCA	GGCCGAGTCT	GAGGCTGAG	TGGCATGAGG	GAGGCAGAGC	GGGTCCCACT	2940
	GTCCCCACAC	TGGCCACGGC	TGTGCAGGTG	TGCCCTGGGC	CCCTAGGGTG	GGGCTCAGCC	3000
30	AGGGGCTGCC	CTCGGCAGGG	TGGGGGATTT	GCCAGCGTGG	CCCTCCCTCC	AGCAGCACCT	3060
	GCCCTGGGCT	GGGCCACGGG	AAGCCCTAGG	AGCCCTTGGG	GACAGACACA	CAGCCCTGTC	3120
	CTCTGTAGGA	GACTGTCCCT	TTCTGTGAGC	GCCCTGTCC	TCCCGACCTC	CATGCCCACT	3180
	CGGGGGCATG	CCTAGTCCAT	GTGCGTAGGG	ACAGGCCCTC	CCTCACCCAT	CTACCCCCAC	3240
	GGCACTAACC	CCTGGCTGCC	CTGCCCAGCC	TCGCACCCGC	ATGGGGACAC	AACCGACTCC	3300
35	GGGGACATGC	ACTCTCGGGC	CCTGTGGAGG	GACTGGTGCA	GATGCCACAC	CACACACTCA	3360
	GCCCAGACCC	GTTCAACAAA	CCCCGCACTG	AGGTTGGCCG	GCCACACGGC	CACCACACAC	3420
	ACACGTGCAC	GCCTCACACA	CGGAGCCTCA	CCCGGGCGAA	CTGCACAGCA	CCCAGACCAG	3480
	AGCAAGGTCC	TGCACACCGT	GAACACTCCT	CGGACACAGG	CCCCACGAG	CCCCACGGG	3540
	CACCTCAAGG	CCCACGAGCC	TCTCGGCAGC	TTCTCCACAT	GCTGACCTGC	TCAGACAAAC	3600
40	CCAGCCCTCC	TCTCACAAGG	GTGCCCCTGC	AGCCGCCACA	CACACACAGG	GGATCACACA	3660
	CCACGTCAAG	TCCCTGGCCC	TGGCCCACTT	CCCAGTGCCG	CCCTTCCCTG	CAGGACGGAT	3720
	CAGCCTCGAC	TGTGCCTTCT	AGTTGCCAGC	CATCTGTTGT	TGCCCCCTCC	CCCGTGCTTT	3780
	CCTTGACCCT	GGAAGGTGCC	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	3840
	CGCATGTGCT	GAGTAGGTGT	CATTCTATT	TGGGGGTGG	GGTGGGGCAG	GACAGCAAGG	3900
45	GGGAGGATTG	GGAAGACAAT	AGCAGGCATG	CTGGGGATGC	GGTGGGCTCT	ATGGCTTCTG	3960
	AGGCGGAAAG	AACCAGCTGG	GGCTCTAGGG	GGTATCCCCA	CGCGCCCTGT	AGCGGCGCAT	4020
	TAAGCGCGGC	GGGTGTGGTG	GTTACGCGCA	GCGTGACCGC	TACACTTGCC	AGCGCCCTAG	4080
	CGCCCGCTCC	TTTCGCTTTC	TTCCCTTCCT	TTCTCGCCAC	GTTCCGCGGG	CCTCTCAAAA	4140
	AAGGGAAAAA	AAGCATGCAT	CTCAATTAGT	CAGCAACCAT	AGTCCCGCCC	CTAACTCCGC	4200
50	CCATCCCGCC	CCTAACTCCG	CCCAGTTCCG	CCCATTCTCC	GCCCCATGGC	TGACTAATTT	4260
	TTTTTATTTA	TGCAGAGGCC	GAGGCGGCCT	CGGCCTCTGA	GCTATTCCAG	AAGTAGTGAG	4320
	GAGGCTTTTT	TGGAGGCCTA	GGCTTTTGCA	AAAAGCTTGG	ACAGCTCAGG	GCTGCGATTT	4380
	CGCGCCAAAC	TTGACGGCAA	TCCTAGCGTG	AAGGCTGGTA	GGATTTTATC	CCCGCTGCCA	4440
	TCATGTTTCG	ACCATGAAC	TGCATCGTGC	CCGTGTCCCA	AAATATGGGG	ATTGGCAAGA	4500
55	ACGGAGACCT	ACCCTGGCCT	CCGCTCAGGA	ACGAGTTCAA	GTAATTCCAA	AGAATGACCA	4560
	CAACCTCTTC	AGTGAAGGT	AAACAGAATC	TGGTGATTAT	GGGTAGGAAA	ACCTGGTTCT	4620
	CCATTCTCTA	GAAGAATCGA	CCTTTAAAGG	ACAGAATTAA	TATAGTTCTC	AGTAGAGAAC	4680
	TCAAAGAACC	ACCACGAGGA	GCTCATTTTC	TTGCCAAAAG	TTGGATGATG	GCCTTAAGAC	4740
	TTATTGAACA	ACCGAATTG	GCAAGTAAAG	TAGACATGGT	TTGATAGTGC	GGAGGCAGTT	4800

	CTGTTTACCA	GGAAGCCATG	AATCAACCAG	GCCACCTTAG	ACTCTTTGTG	ACAAGGATCA	4860
	TGCAGGAATT	TGAAAGTGAC	ACGTTTTTCC	CAGAAATTGA	TTTGGGGAAA	TATAAACTTC	4920
	TCCCAGAATA	CCCAGGCGTC	CTCTCTGAGG	TCCAGGAGGA	AAAAGGCATC	AAGTATAAGT	4980
	TTGAAGTCTA	CGAGAAGAAA	GACTAACAGG	AAGATGCTTT	CAAGTTCTCT	GCTCCCTTCC	5040
5	TAAAGCTATG	CATTTTTATA	AGACCATGGG	ACTTTTGCTG	GCTTTAGATC	TCTTTGTGAA	5100
	GGAACCTTAC	TTCTGTGGTG	TGACATAATT	GGACAACTA	CCTACAGAGA	TTTAAAGCTC	5160
	TAAGGTAAAT	ATAAAATTTT	TAAGTGTATA	ATGTGTTAAA	CTACTGATTC	TAATTGTTTG	5220
	TGTATTTTAG	ATTCCAACCT	ATGGAACCTG	TGAATGGGAG	CAGTGGTGGG	ATGCCCTTAA	5280
	TGAGGAAAAC	CTGTTTGTCT	CAGAAGAAAT	GCCATCTAGT	GATGATGAGG	CTACTGCTGA	5340
10	CTCTCAACAT	TCTACTCCTC	CAAAAAAGAA	GAGAAAGGTA	GAAGACCCCA	AGGACTTTCC	5400
	TTCAGAATTG	CTAAGTTTTT	TGAGTCATGC	TGTGTTTAGT	AATAGAACTC	TTGCTTGCTT	5460
	TGCTATTTAC	ACCACAAAGG	AAAAAGCTGC	ACTGCTATAC	AAGAAAATTA	TGGAAAAATA	5520
	TTCTGTAACC	TTTATAAGTA	GGCATAACAG	TTATAATCAT	AACATACTGT	TTTTTCTTAC	5580
	TCCACACAGG	CATAGAGTGT	CTGCTATTAA	TAACTATGCT	CAAAAATTGT	GTACCTTTAG	5640
15	CTTTTTTAAT	TGTAAAGGGG	TTAATAAGGA	ATATTTGATG	TATAGTGCTT	TGACTAGAGA	5700
	TCATAATCAG	CCATACCACA	TTTGTAGAGG	TTTTACTTGC	TTTAAAAAAC	CTCCACACAC	5760
	TCCCCCTGAA	CCTGAAACAT	AAATGAATG	CAATTGTTGT	TGTTAACTTG	TTTATTGCAG	5820
	CTTATAATGG	TTACAAATAA	AGCAATAGCA	TCACAAATTT	CACAAATAAA	GCATTTTTTT	5880
	CACGTGCATT	TAGTTGTGGT	TTGTCCAAAC	TCATCAATGT	ATCTTATCAT	GTCTGGATCG	5940
20	GCTGGATGAT	CCTCCAGCGC	GGGGATCTCA	TGCTGGAGTT	CTTCGCCCCAC	CCCAACTTGT	6000
	TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG	6060
	CATTTTTTTT	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG	6120
	TCTGTATACC	GTCGACCTCT	AGCTAGAGCT	TGGCGTAATC	ATGGTCATAG	CTGTTTCTCT	6180
	TGTGAAATGG	TTATCCGCTC	ACAAATCCAC	ACAACATACG	AGCCGGAAGC	ATAAAGTGTA	6240
25	AAGCCTGGGG	TGCCTAATGA	GTGAGCTAAC	TCACATTAAT	TGCGTTGCGC	TCACTGCCCG	6300
	CTTTCAGTC	GGGAAACCTG	TCGTGCCAGC	TGCATTAAAT	AATCGGCCAA	CGCGCGGGGA	6360
	GAGGCGGTTT	GCGTATTGGG	CGCTCTTCCG	CTTCCTCGCT	CACTGACTCG	CTGCGCTCGG	6420
	TCGTTCCGGT	GCGGCGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATACGG	TTATCCACAG	6480
	AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	6540
30	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCCTGAC	GAGCATCACA	6600
	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	6660
	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCGCGTT	ACCGGATACC	6720
	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	6780
	TCAGTTCCGT	GTAGGTGCTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	6840
35	CCGACCCTG	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	6900
	TATCGCCACT	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	6960
	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTGGTGA	7020
	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG	GA AAAAGAGT	TGGTAGCTCT	TGATCCGGCA	7080
	AACAAACCAC	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	7140
40	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAAAC	7200
	AAAATCAGC	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	7260
	TTTTAAATTA	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	7320
	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCT	7380
	CCATAGTTGC	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	7440
45	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	7500
	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCTCCA	7560
	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCGCCAGTT	AATAGTTTGC	7620
	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT	7680
	CATTACAGCT	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	7740
50	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTAT	7800
	CACCTCATGG	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	7860
	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	7920
	GTTGCTCTTG	CCCGCGGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAAG	7980
	TGCTCATCAT	TGGAAAACGT	TCTTCGGGGC	GAAAACTCTC	AAGGATCTTA	CCGCTGTTGA	8040
55	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC	CCAACTGATC	TTCAGCATCT	TTTACTTTCA	8100
	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAGG	GGAATAAGGG	8160
	CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	AGCATTATATC	8220
	AGGGTTATTG	TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	8280
	GGGTTCCGCG	CACATTTCCT	CGAAAAGTGC	CACCTGACGT	CCBRAAG		8327

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 8897 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGCAC CATGAAGTTG CCTGTTAGGC 60
15 TGTTGGTGCT GATGTTCTGG ATTCTGCTT CCAGCAGTGA TGTTTGTATG ACCCAAATTC 120
CAGTCTCCCT GCCTGTCACT CTGGGAGATC AAGCGTCCAT CTCTGCAGA TCTAGTCAGA 180
TCATTGTACA TAATAATGGC AACACCTATT TAGAATGGTA CCTGCAGAAA CCAGGCCAGT 240
CTCCACAGCT CCTGATCTAC AAAGTTTCCA ACCGATTTTC TGGGGTCCCA GACAGGTTCA 300
GCGGCAGTGG ATCAGGGACA GATTTACAC TCAAGATCAG CAGAGTGGAG GCTGAGGATC 360
20 TGGGAGTTTA TTACTGCTTT CAAGGTTTAC ATGTTCCATT CACGTTTCGC TCGGGGACAA 420
AGTTGGAAT AAAACGTAAG TCTCGAGTCT CTAGATAACC GGTCAATCGA TTGGAATTCT 480
AAACTCTGAG GGGGTGCGAT GACGTGGCCA TTCTTGCCT AAAGCATTGA GTTTACTGCA 540
AGGTGAGAAA AGCATGCAAA GCCCTCAGAA TGGCTGCAAA GAGCTCCAAC AAAACAATTT 600
AGAACTTTAT TAAGGAATAG GGGGAAGCTA GGAAGAACT CAAAACATCA AGATTTTAAA 660
25 TACGCTTCTT GGTCTCCTTG CTATAATTAT CTGGGATAAG CATGCTGTTT TCTGTCTGTC 720
CCTAACATGC CCTTATCCGC AAACAACACA CCCAAGGGCA GAACTTTGTT ACTTAAACAC 780
CATCTGTTT GCTTCTTTCC TCAGGAACTG TGGCTGCACC ATCTGTCTTC ATCTTCCCGC 840
CATCTGATGA GCAGTTGAAA TCTGGAAGT CCTCTGTTGT GTGCTGCTG AATAACTTCT 900
ATCCAGAGA GGCCTAAGTA CAGTGAAGG TGGATAACGC CCTCCAATCG GGTAACTCCC 960
30 AGGAGAGTGT CACAGAGCAG GAGAGCAAGG ACAGCACCTA CAGCCTCAGC AGCACCCCTGA 1020
CGCTGAGCAA AGCAGACTAC GAGAAACACA AAGTCTACGC CTGCGAAGTC ACCCATCAGG 1080
GCCTGAGCTC GCCGTCACA AAGAGCTTCA ACAGGGGAGA GTGTTAGAGG GAGAAGTGCC 1140
CCCACCTGCT CCTCAGTTCC AGCCTGACCC CCTCCCATCC TTTGGCCTCT GACCCCTTTT 1200
CCACAGGGGA CCTACCCCTA TTGGGTCCT CAGCTCATC TTTCACCTCA CCCCCTCCT 1260
35 CCTCCTTGGC TTTAATTATG CTAATTGTTG AGGAGAATGA ATAAATAAAG TGAATCTTTG 1320
CACCTGTGGT TTCTCTCTTT CCTCATTATA TAATTATTAT CTGTTGTTT ACCAACTACT 1380
CAATTTCTCT TATAAGGGAC TAAATATGTA GTCATCTTAA GGCACGTAAC CATTATATAA 1440
AATCATCTCT CATTCTATTT TACCCTATCA TCCTCTGCAA GACAGTCTC CCTCAAACCC 1500
ACAAGCCTTC TGTCTCACA GTCCCTTGGG CCATGTTAGG AGAGACTTGC TTCCTTGTTT 1560
40 TCCCCTCCTC AGCAAGCCCT CATAGTCTTT TTTAAGGGTG ACAGGTCTTA CAGTCATATA 1620
TCCTTTGATT CAATTCCTG AGAATCAACC AAAGCAAATT TTTCAAAGA AGAAACCTGC 1680
TATAAAGAGA ATCATTCATT GCAACATGAT ATAAATAAAC AACACAATAA AAGCAATTAA 1740
ATAAACAAAC AATAGGGAAT TGTTAAGTT CATCATGGTA CTTAGACTTA ATGGAATGTC 1800
ATGCCTTATT TACATTTTAA AACAGGTACT GAGGGACTCC TGTCTGCCAA GGGCCGTATT 1860
45 GAGTACTTTC CACAACCTAA TTTAATCCAC ACTATACTGT GAGATTAAAA ACATTCATTA 1920
AAATGTTGCA AAGGTTCTAT AAAGCTGAGA GACAAATATA TTCTATAACT CAGCAATCCC 1980
ACTTCTAGAT GACTGAGTGT CCCCAACCCAC CAAAAAATA TGCAAGAATG TTCAAAGCAG 2040
CTTTATTAC AAAAGCCAAA AATTGGAAAT AGCCCGATTG TCCAACAATA GAATGAGTTA 2100
TTAAACTGTG GTATGTTTAT ACATTAGAAT ACCCAATGAG GAGAATTAAC AAGCTACAAC 2160
50 TATACCTACT CACACAGATG AATCTCATAA AAATAATGTT ACATAAGAGA AACTCAATGC 2220
AAAAGATATG TTCTGTATGT TTTCAATCCAT ATAAAGTTCA AAACCAGGTA AAAATAAAGT 2280
TAGAAATTTG GATGGAAATT ACTCTTAGCT GGGGGTGGGC GAGTTAGTGC CTGGGAGAAG 2340
ACAAGAAGGG GCTTCTGGGG TCTTGTAAT GTTCTGTTCC TCGTGTGGGG TTGTGCAATT 2400
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55 TCTTATACCC AGTTAATAGA TAGAAGAGGA ATAAGTAATA GGTCAGACC AACGCAGCTG 2520
GTAAGTGGGG GCCTGGGATC AAATAGCTAC CTGCCTAATC CTGCCWCTT GAGCCCTGAA 2580
TGAGTCTGCC TTCCAGGGCT CAAGGTGCTC AACAAACAA CAGGCTGCT ATTTTCCTGG 2640
CATCTGTGCC CTGTTTGCT AGCTAGGAGC ACACATACAT AGAAATTAAA TGAACAGAC 2700
CTTCAGCAAG GGGACAGAGG ACAGAAATTA CCTTGCCAG AACTGGAAA CCCATGTATG 2760

	AACACTCACA	TGTTTGGGAA	GGGGGAAGGG	CACATGTAAA	TGAGGACTCT	TCCTCATTCT	2820
	ATGGGGCACT	CTGGCCCTGC	CCCTCTCAGC	TACTCATCCA	TCCAACACAC	CTTTCTAAGT	2880
	ACCTCTCTCT	GCCTACACTC	TGAAGGGGTT	CAGGAGTAAC	TAACACAGCA	TCCCTTCCCT	2940
	CAATGACTG	ACAATCCCTT	TGTCCTGCTT	TGTTTTCTT	TCCAGTCAGT	ACTGGGAAAG	3000
5	TGGGGAAGGA	CAGTCATGGA	GAAACTACAT	AAGGAAGCAC	CTTGCCCTTC	TGCCTCTTGA	3060
	GAATGTTGAT	GAGTATCAAA	TCTTTCAAAC	TTTGAGGTT	TGAGTAGGGG	TGAGACTCAG	3120
	TAATGTCCCT	TCCAATGACA	TGAACCTGCT	CACCTATCCC	TGGGGGCCAA	ATTGAACAA	3180
	CAAAGGCAGG	CATAATCCAG	TTATGAATTC	TTGCGGCCGC	TTGCTAGCTT	CACGTGTTGG	3240
	ATCCAACCGG	GGAAGGGCCC	TATTCTATAG	TGTCACCTAA	ATGCTAGAGC	TCGCTGATCA	3300
10	GCCTCGACTG	TGCCTTCTAG	TTGCCAGCCA	TCTGTTGTTT	GCCCCCTCCC	CGTGCTTCC	3360
	TTGACCCTGG	AAGGTGCCAC	TCCCCTGTC	CTTTCCTAAT	AAAATGAGGA	AATTGCATCG	3420
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	GAGGATTGGG	AAGACAATAG	CAGGCATGCT	GGGGATGCGG	TGGGCTCTAT	GGCTTCTGAG	3540
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15	AGCGCGCGG	GTGTGGTGGT	TACGCGCAGC	GTGACCGCTA	CACCTGCCAG	CGCCCTAGCG	3660
	CCCGCTCCTT	TCGCTTTCTT	CCCTTCTTTT	CTCGCCACGT	TCGCGGGGCC	TCTCAAAAAA	3720
	GGGAAAAAAA	GCATGCATCT	CAATTAGTCA	GCAACCATAG	TCCCGCCCCC	AACTCCGCC	3780
	ATCCCGCCCC	TAACCTCCGC	CAGTTCGCC	CATTCTCCGC	CCCATGGCTG	ACTAATTTT	3840
	TTTATTTATG	CAGAGGCCGA	GGCGCCTCG	GCCTCTGAGC	TATTCAGAA	GTAGTGAGGA	3900
20	GGCTTTTGTG	GAGGCCCTAG	CTTTTGCAAA	AAGCTTGGAC	AGCTCAGGGC	TGCGATTTCG	3960
	CGCCAAACTT	GACGGCAATC	CTAGCGTGAA	GGCTGGTAGG	ATTTTATCCC	CGCTGCCATC	4020
	ATGTTTCGAC	CATTGAACTG	CATCGTCGCC	GTGTCCCAAA	ATATGGGGAT	TGGCAAGAAC	4080
	GGAGACCTAC	CCTGGCCTCC	GCTCAGGAAC	GAGTTCAGT	ACTTCCAAAG	AATGACCACA	4140
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25	ATTCCTGAGA	AGAATCGACC	TTTAAAGGAC	AGAATTAATA	TAGTTCTCAG	TAGAGAACTC	4260
	AAAGAACCAC	CACGAGGAGC	TCATTTTCTT	GCCAAAAGTT	TGGATGATGC	CTTAAGACTT	4320
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	GTTTACCAGG	AAGCCATGAA	TCAACCAGGC	CACCTTAGAC	TCTTTGTGAC	AAGGATCATG	4440
	CAGGAATTTG	AAAGTGACAC	GTTTTTCCCA	GAAATTGATT	TGGGGAAATA	TAAACTTCTC	4500
30	CCAGAATACC	CAGGCGTCTT	CTCTGAGGTC	CAGGAGGAAA	AAGGCATCAA	GTATAAGTTT	4560
	GAACTCTACG	AGAAGAAAGA	CTAACAGGAA	GATGCTTTCA	AGTTCTCTGC	TCCCCTCCTA	4620
	AAGCTATGCA	TTTTTATAAG	ACCATGGGAC	TTTTGCTGGC	TTTAGATCTC	TTTGTGAAGG	4680
	AACCTTACTT	CTGTGGTGTG	ACATAATTGG	ACAACTACC	TACAGAGATT	TAAAGCTCTA	4740
	AGGTAAATAT	AAAATTTTAA	AGTGATAAAT	GTGTTAAACT	ACTGATTCTA	ATTGTTTGTG	4800
35	TATTTTAGAT	TCCAACCTAT	GGAAGTATG	AATGGGAGCA	GTGGTGGAA	GCCTTTAATG	4860
	AGGAAAACCT	TTTTTGCTCA	GAAGAAATGC	CATCTAGTGA	TGATGAGGCT	ACTGCTGACT	4920
	CTCAACATTC	TACTCTCCA	AAAAAGAAGA	GAAAGGTAGA	AGACCCCAAG	GACTTTCTCT	4980
	CAGAATTGCT	AAGTTTTTTG	AGTCATGCTG	TGTTTAGTAA	TAGAACTCTT	GCTTGCTTTG	5040
	CTATTTACAC	CACAAAGGAA	AAAGCTGCAC	TGCTATACAA	GAAATATTATG	GAAAAATATT	5100
40	CTGTAACCTT	TATAAGTAGG	CATAACAGTT	ATAATCATAA	CATACTGTTT	TTTCTTACTC	5160
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	TTTTAATTG	TAAAGGGGTT	AATAAGGAAT	ATTGATGTA	TAGTGCCCTG	ACTAGAGATC	5280
	ATAATCAGCC	ATACCACATT	TGTAGAGGTT	TTACTTGCTT	TAAAAACCT	CCCACACCTC	5340
	CCCCTGAACC	TGAAACATAA	AATGAATGCA	ATTGTTGTTG	TTAACTTGTT	TATTGCAGCT	5400
45	TATAATGGTT	ACAAATAAAG	CAATAGCATC	ACAAATTTC	CAATAAAGC	ATTTTTTTCA	5460
	CTGCATTCTA	GTGTGGTTT	GTCCAACTC	ATCAATGTAT	CTTATCATGT	CTGGATCGGC	5520
	TGGATGATCC	TCCAGCGCGG	GGATCTCATG	CTGGAGTTCT	TCGCCCACCC	CAACTTGTTT	5580
	ATTGCAGCTT	ATAATGGTTA	CAATAAAGC	AATAGCATCA	CAAAATTCAC	AAATAAAGCA	5640
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	TGAAATTGTT	ATCCGCTCAC	AATTCACAC	AACATACGAG	CCGGAAGCAT	AAAGTGTA	5820
	GCCTGGGGTG	CCTAATGAGT	GAGCTAACTC	ACATTAATTG	CGTTGCGCTC	ACTGCCCGCT	5880
	TTCCAGTCGG	GAAACCTGTC	GTGCCAGCTG	CATTAATGAA	TCGGCCAACG	CGCGGGGAGA	5940
	GGCGGTTTGC	GTATTGGGCG	CTCTCCGCT	TCTCGCTCA	CTGACTCGCT	GCGCTCGGTC	6000
55	GTTCCGCTGC	GGCGAGCGGT	ATCAGCTCAC	TCAAAGGCGG	TAATACGGTT	ATCCACAGAA	6060
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	AAAAAGGCCG	CGTTGCTGGC	GTTTTTCCAT	AGGCTCCGCC	CCCCTGACGA	GCATCACAAA	6180
	AATCAGCGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	CCAGGCGTTT	6240
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 5 ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC 6600
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 AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCTTG CAACTTTATC CGCCTCCATC 7140
 15 CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGGCG 7200
 AACGTGTGTT CCATTGCTAC AGGCATCGTG GTGTACGCT CGTCGTTTGG TATGGCTTCA 7260
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 25 ACACGGAAT GTTGAATACT CATACTCTTC CTTTTCATAT ATTATTGAAG CATTATCAG 7800
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 30 TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG TATCTGCTCC CTGCTTGTGT 8100
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 35 GCCTGGCTGA CCGCCCAACG ACCCGCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT 8400
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 40 CAATGGGCGT GGATAGCGGT TTGACTCACG GGGATTTCCT AGTCTCCACC CCATTGACGT 8700
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 CGCCCCATTG ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC 8820
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 TAGGGAGACC CAAGCTT 8897

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8321 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60
 TTGGAATTCT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG TGGTTAAGCT TGGTCTTCCT 120

	TGTCCTTGTT	TTAAAAGGTG	TCCAGTGTGA	AGTGCAACTG	GTGGAGTCTG	GGGGAGGCTT	180
	AGTGCAGCCT	GGAGGGTCCC	TGCGACTTTC	CTGTGCTGCA	TCTGGATTCC	CGTTCAGTGA	240
	CTATTACATG	TATTGGGTTT	GCCAGGCTCC	AGGCAAGGGA	CTGGAGTGGG	TCTCATACAT	300
	TAGTCAAGAT	GGTGATATAA	CCGACTATGC	AGACTCCGTA	AAGGGTCGAT	TCACCATCTC	360
5	CAGAGACAAT	GCAAAGAACA	GCCTGTACCT	GCAAATGAAC	AGCCTGAGGG	ACGAGGACAC	420
	AGCCGTGTAT	TACTGTGCAA	GAGGCCTGGC	GGACGGGGCC	TGGTTTGCTT	ACTGGGGCCA	480
	AGGGAAGTCTG	GTCACGGTCT	CTTCCGCTAG	CACCAAGGGC	CCATCGGTCT	TCCCCCTGGC	540
	ACCTCTCTCC	AAGAGCACCT	CTGGGGGCAC	AGCGGCCCTG	GGCTGCCTGG	TCAAGGACTA	600
	CTTCCCCGAA	CCGGTGACGG	TGTCGTGGAA	CTCAGGCGCC	CTGACCAGCG	GCGTGCACAC	660
10	CTTCCCCGCT	GTCCTACAGT	CCTCAGGACT	CTACTCCCTC	AGCAGCGTGG	TCACCGTGCC	720
	CTCCAGCAGC	TTGGGCACCC	AGACCTACAT	CTGCAACGTG	AATCAACAAG	CCAGCAACAC	780
	CAAGGTGGAC	AAGAAAGTTG	GTGAGAGGCC	AGCACAGGGA	GGGAGGGTGT	CTGCTGGAAG	840
	CCAGGCTCAG	CGCTCCTGCC	TGGACGCATC	CCGGCTATGC	AGCCCCAGTC	CAGGGCAGCA	900
	AGGCAGGCC	CGTCTGCCTC	TTCAACCGGA	GGCCTCTGCC	CGCCCCACTC	ATGCTCAGGG	960
15	AGAGGGTCTT	CTGGCTTTTT	CCCCAGGCTC	TGGGCAGGCA	CAGGCTAGGT	GCCCCCTAAC	1020
	CAGGCCCTGC	ACACAAAGGG	GCAGGTGCTG	GGCTCAGACC	TGCCAAGAGC	CATATCCGGG	1080
	AGGACCCTGC	CCCTGACCTA	AGCCCCCCCC	AAAGGCCAAA	CTCTCCACTC	CCTCAGCTCG	1140
	GACACCTTCT	CTCCTCCACG	ATTCCAGTAA	CTCCCAATCT	TCTCTCTGCA	GAGCCCCAAT	1200
	CTTGTGACAA	AATCAACACA	TGCCCCACGT	CCCCAGGTAA	GCCAGCCCAG	GCCTCGCCCT	1260
20	CCAGCTCAAG	GCGGGACAGG	TGCCCTAGAG	TAGCCTGCAT	CCAGGGACAC	ACCACGTGGG	1320
	TACCAACATG	TCCGGAGCCA	CATGGACAGA	GGCCGGCTCG	GCCCCACCCTC	TGCCCTGAGA	1380
	GTGACCGCTG	TACCAACCTC	TGTCCCTACA	GGGCAGCCCC	GAGAACCACA	GGTGTACACC	1440
	GTGCCCCCAT	CCCCGGATGA	GCTGACCAAG	AACCAGGTCA	GCCTGACCTG	CCTGGTCAAA	1500
	GGCTTCTATC	CCAGCGACAT	CGCCGTGGAG	TGGGAGAGCA	ATGGGCAGCC	GGAGAACAAC	1560
25	TACAGACCA	CGCCTCCCGT	GCTGGACTCC	GACGGCTCCT	TCTTCTCTTA	CAGCAAGCTC	1620
	ACCGTGGACA	AGAGCAGGTG	GCAGCAGGGG	AACGTCTTCT	CATGTCTCCG	GATGCATGAG	1680
	GCTCTGCACA	ACCACTACAC	GCAGAAGAGC	CTCTCCCTGT	CTCCGGGTAA	ATGAGTGGCA	1740
	CGGCCGGCAA	GCCCCCGCTC	CGCGGTGCGA	CGAGGATGCT	TGGCAGCTAC		1800
	CCCTGTACAC	TACTTCCCGG	GCGCCCAGCA	TGGAATAAAA	GCACCACGCG	CTGCCCTGGG	1860
30	CCCCTGCGAG	ACTGTGATGG	TTCTTTCCAC	GGGTCAAGCC	GAGTCTGAGG	CCTGAGTGGC	1920
	ATGAGGGAGG	CAGAGCGGGT	CCCACTGTCC	CCACACTGGC	CCAGGCTGTG	CAGGTGTGCC	1980
	TGGGGCCCCCT	AGGGTGGGGC	TCAGCCAGGG	GCTGCCCTCG	GCAGGGTGGG	GGATTGCGCA	2040
	GCGTGGCCCT	CCCTCCAGCA	GCACCTGCC	TGGGCTGGGC	CACGGGAAGC	CCTAGGAGCC	2100
	CCTGGGGACA	GACACACAGC	CCCTGCCTCT	GTAGGAGACT	GTCCTGTCTT	GTGAGCGCCC	2160
35	CTGTCTCTCC	GACCTCCATG	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	2220
	GCCCTCCCTC	ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGCCCTGC	CCAGCCTCGC	2280
	ACCCGCGATG	GGACACAACC	GACTCGGGGG	ACATGCACTC	TCGGGCCCTG	TGGAGGGACT	2340
	GGTGCAGATG	CCCACACACA	CACCTAGCCC	AGACCCGTTT	AACAAACCCC	GCACTGAGGT	2400
	TGGCCGGCCA	CACGGCCACC	ACACACACAC	GTGCACGCCT	CACACACGGA	GCCTCACCCG	2460
40	GGCGAACTGC	ACAGCACCCA	GACCAGAGCA	AGGTCCCTCG	ACACGTGAAC	ACTCCTCGGA	2520
	CACAGGCCCC	CACGAGCCCC	ACGCGGCACC	TCAAGGCCCA	CGAGCCTCTC	GGCAGCTTCT	2580
	CCACATGCTG	ACCTGCTCAG	ACAAACCCAG	CCCTCCTCTC	ACAAGGGTGC	CCCTGCAGCC	2640
	GCCACACACA	CACAGGGGAT	CACACACCAC	GTCAAGTCCC	TGGCCCTGGC	CCACTTCCCA	2700
	GTGCCGCCCT	TCCCTGCAGG	ACGGATCAGC	CTCGACTGTG	CCTTCTAGTT	GCCAGCCATC	2760
45	TGTTGTTTGC	CCCTCCCCCG	TGCCTTCCCT	GACCCTGGAA	GGTGCCACTC	CCACTGTCTT	2820
	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCTAT	CTATTCTGGG	2880
	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	GGCATGCTGG	2940
	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAAC	AGCTGGGGCT	CTAGGGGGTA	3000
	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG	CGCGGCGGGT	GTGGTGGTTA	CGCGCAGCGT	3060
50	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	CTTCTTTTCT	3120
	CGCCACGTTT	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	ATTAGTCAGC	3180
	AACCATAGTC	CGCCCCCTAA	CTCCGCCCAT	CCCGCCCCTA	ACTCCGCCCA	GTTCCGCCCA	3240
	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	CGCCTCGGC	3300
	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGA	GGCCTAGGCT	TTTGCAAAAA	3360
55	GCTTGGACAG	CTCAGGCTG	CGATTTGCGG	CCAAACTTGA	CGGCAATCCT	AGCGTGAAGG	3420
	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTCGACCA	TTGAACTGCA	TCGTCCCGCT	3480
	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	TCAGGAACGA	3540
	GTTCAAGTAC	TTCCAAAGAA	TGACCAACAAC	CTCTTCAGTG	GAAGGTAAAC	AGAATCTGGT	3600
	GATTATGGGT	AGGAAAACCT	GGTTCTCCAT	TCCTGAGAAG	AATCGACCTT	TAAAGGACAG	3660

	AAATTAATATA	GTTCTCAGTA	GAGAACTCAA	AGAACCACCA	CGAGGAGCTC	ATTTTCTTGC	3720
	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	GTAAAGTAGA	3780
	CATGGTTTGG	ATAGTCGGAG	GCAGTTCTGT	TTACCAGGAA	GCCATGAATC	AACCAGGCCA	3840
	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	TTTTCCAGA	3900
5	AATTGATTTG	GGGAAATATA	AACTTCTCCC	AGAATACCCA	GGCGTCTCT	CTGAGGTCCA	3960
	GGAGGAAAAA	GGCATCAAGT	ATAAGTTTGA	AGTCTACGAG	AAGAAAGACT	AACAGGAAGA	4020
	TGCTTTCAAG	TTCTCTGCTC	CCCTCCTAAA	GCTATGCATT	TTTATAAGAC	CATGGGACTT	4080
	TTGCTGGCTT	TAGATCTCTT	TGTGAAGGAA	CCTTACTTCT	GTGGTGTGAC	ATAATTGGAC	4140
	AAACTACCTA	CAGAGATTTA	AAGCTCTAAG	GTAAATATAA	AATTTTAAAG	TGTATAATGT	4200
10	GTTAAACTAC	TGATTCTAAT	TGTTTGTGTA	TTTTAGATT	CAACCTATGG	AACTGATGAA	4260
	TGGGAGCAGT	GGTGGAAATG	CTTTAATGAG	GAAAACCTGT	TTTGCTCAGA	AGAAATGCCA	4320
	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	AAAGAAGAGA	4380
	AAGGTAGAAG	ACCCCAAGGA	CTTTCCTTCA	GAATTGCTAA	GTTTTTGAG	TCATGCTGTG	4440
	TTTAGTAATA	GAACCTTTCG	TTGCTTTGCT	ATTTACACCA	CAAAGGAAAA	AGCTGCACCTG	4500
15	CTATACAAGA	AAATTATGGA	AAAAATTCT	GTAACCTTTA	TAAGTAGGCA	TAACAGTTAT	4560
	AATCATAACA	TACTGTTTTT	TCTTACTCCA	CACAGGCATA	GAGTGTCTGC	TATTAATAAC	4620
	TATGCTCAAA	AATTGTGTAC	CTTTAGCTTT	TTAATTTGTA	AAGGGGTAA	TAAGGAATAT	4680
	TGATGTATA	GTGCCCTGAC	TAGAGATCAT	AATCAGCCAT	ACCACATTG	TAGAGGTTTT	4740
	ACTTGCTTTA	AAAAACCTCC	CACACCTCCC	CCTGAACCTG	AAACATAAAA	TGAATGCAAT	4800
20	TGTTGTGTTT	AACCTGTTTA	TTGCAGCTTA	TAATGGTTAC	AAATAAAGCA	ATAGCATCAC	4860
	AAATTTTACA	AAATAAGCAT	TTTTTTCAC	GCATTCTAGT	TGTGGTTTGT	CCAAACTCAT	4920
	CAATGTATCT	TATCATGTCT	GGATCGGCTG	GATGATCCTC	CAGCGCGGGG	ATCTCATGCT	4980
	GGAGTTCTTC	GCCCAACCCA	ACTTGTTTAT	TGCAGCTTAT	AATGGTTACA	AATAAAGCAA	5040
	TAGCATCACA	AATTTTCAAA	ATAAAGCAT	TTTTTCACTG	CATTCTAGTT	GTGGTTTGTG	5100
25	CAAACTCATC	AATGTATCTT	ATCATGTCTG	TATACCGTCG	ACCTCTAGCT	AGAGCTTGGC	5160
	GTAATCATGG	TCATAGCTGT	TTCTGTGTG	AAATTGTTAT	CCGCTCACAA	TTCCACACAA	5220
	CATACGAGCC	GGAAGCATAA	AGTGTAAAGC	CTGGGGTGCC	TAATGAGTGA	GCTAACTCAC	5280
	ATTAATTGCG	TGCGGCTCAC	TGCGCGCTTT	CCAGTCGGGA	AACCTGTCTG	GCCAGCTGCA	5340
	TTAATGAATC	GGCCAACGCG	CGGGGAGAGG	CGGTTTGCCT	ATTGGGCGCT	CTTCGCTTC	5400
30	CTCGCTCACT	GACTCGCTGC	GCTCGGTCTG	TCGGCTGCGG	CGAGCGGTAT	CAGCTCACTC	5460
	AAAGGCGGTA	ATACGGTTAT	CCACAGAATC	AGGGGATAAC	GCAGGAAAGA	ACATGTGAGC	5520
	AAAAGGCCAG	CAAAGGCCA	GGAACCGTAA	AAAGGCGCGG	TTGCTGGCGT	TTTTCCATAG	5580
	GCTCCGCCCC	CCTGACGAGC	ATCACAAAAA	TCGACGCTCA	AGTCAGAGGT	GGCGAAACCC	5640
	GACAGGACTA	TAAAGATACC	AGGCGTTTCC	CCCTGGAAGC	TCCCTCGTGC	GCTCTCCTGT	5700
35	TTCCGACCTG	CCGCTTACCG	GATACCTGTC	CGCCTTCTC	CCTTCGGGAA	GCCTGGCGCT	5760
	TTCTCAATGC	TCACGCTGTA	GGTATCTCAG	TTCCGGTGTAG	GTCGTTCTGCT	CCAAGCTGGG	5820
	CTGTGTGCAC	GAACCCCCCG	TTCAGCCCGA	CCGCTGCGCC	TTATCCGGTA	ACTATCGTCT	5880
	TGAGTCCAAC	CCGGTAAGAC	ACGACTTATC	GCCACTGGCA	GCAGCCACTG	GTAAACAGGAT	5940
	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC	CTAATACGG	6000
40	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG	AAGCCAGTTA	CCTTCGGAAA	6060
	AAGAGTTGGT	AGCTCTTGAT	CCGGCAAACA	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	6120
	TTGCAAGCAG	CAGATTACGC	GCAGAAAAAA	AGGATCTCAA	GAAGATCCTT	TGATCTTTTC	6180
	TACGGGGTCT	GACGCTCAGT	GGAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT	6240
	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATTAAAAA	TGAAGTTTAA	AATCAATCTA	6300
45	AAGTATATAT	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT	6360
	CTCAGCGATC	TGTCTATTTC	GTTTCATCCAT	AGTTGCCTGA	CTCCCGCTCG	TGTAGATAAC	6420
	TACGATACGG	GAGGGCTTAC	CATCTGGCCC	CAGTGTGCTG	ATGATACCGC	GAGACCCACG	6480
	CTCACCGGCT	CCAGATTTAT	CAGCAATAAA	CCAGCCAGCC	GGAAGGGCCG	AGCGCAGAAG	6540
	TGGTCTCTGA	ACTTTATCCG	CCTCCATCCA	GTCTATTAA	TGTTGCCGGG	AAGCTAGAGT	6600
50	AGTAGTTTCG	CCAGTTAATA	GTTTGCACAA	CGTTGTTGCC	ATTGCTACAG	GCATCGTGGT	6660
	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT	CAAGGCGAGT	6720
	TACATGATCC	CCCATGTTGT	GCAAAAAAGC	GGTTAGCTCC	TTCCGGTCTC	CGATCGTTGT	6780
	CAGAAGTAAG	TTGGCCGCAG	TGTTATCACT	CATGGTTATG	GCAGCACTGC	ATAATTCTCT	6840
	TACTGTCTATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	6900
55	CTGAGAATAG	TGTATGCGGC	GACCGAGTTG	CTCTTGCCCG	GCGTCAATAC	GGGATAATAC	6960
	CGCGCCACAT	AGCAGAACTT	TAAAGTGCT	CATCATTGGA	AAACGTTCTT	CGGGGCGAAA	7020
	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG	TAACCCACTC	GTGACCCCAA	7080
	CTGATCTTCA	GCATCTTTTA	CTTTCACCAG	CGTTTCTGGG	TGAGCAAAAA	CAGGAAGGCA	7140
	AAATGCCGCA	AAAAAGGGAA	TAAGGGCGAC	ACGGAAATGT	TGAATACTCA	TACTCTTCCT	7200

TTTTCAATAT TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA 7260
 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCGGAA AAGTGCCACC 7320
 TGACGTCGAC GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGGCGCGGCT TCGAATAGCC 7380
 AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTT GAGATGGAGT TTGGCGCCGA 7440
 5 TCTCCCGATC CCTATGGTC GACTCTCAGT ACAATCTGCT CTGATGCCGC ATAGTTAAGC 7500
 CAGTATCTGC TCCCTGCTTG TGTGTTGGAG GTCGCTGAGT AGTGCGCGAG CAAAATTTAA 7560
 GCTACAACAA GGCAAGGCTT GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTAGGCGT 7620
 TTTGCGCTGC TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT 7680
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 10 ACATAACTTA CGGTAAATGG CCCGCTGGC TGACCGCCCA ACGACCCCG CCCATTGACG 7800
 TCAATAATGA CGTATGTTCC CATAGTAACG CCAATAGGGA CTTTCCATTG ACGTCAATGG 7860
 GTGGACTATT TACGGTAAAC TGCCCACTTG GCAGTACATC AAGTGATCA TATGCCAAGT 7920
 ACGCCCCCTA TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG 7980
 ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC TATTACCATG 8040
 15 GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGGATAGC GGTGAGTAC ACGGGGATT 8100
 CCAAGTCTCC ACCCCATTGA CGTCAATGGG AGTTTGTGTTT GGCACCAAAA TCAACGGGAC 8160
 TTTCCAAAT GTCGTAACAA CTCCGCCCA TTGACGCAA TGGGCGGTAG GCGTGTACGG 8220
 TGGGAGGTCT ATATAAGCAG AGCTCTCTGG CTAAGTAGAG AACCCACTGC TTAGTGGCTT 8280
 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT G 8321

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8897 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GACGGATCGG GAGATCTGCT AGCCCGGGTG ACCTGAGGCG CGCCGGCTTC GAATAGCCAG 60
 AGTAACCTTT TTTTFTAATT TTATTTTATT TTATTTTGA GATGGAGTTT GGCGCCGATC 120
 35 TCCCGATCCC CTATGGTCGA CTCTCAGTAC AATCTGCTCT GATGCCGAT AGTTAAGCCA 180
 GTATCTGCTC CTGCTTGTG TGTGGAGGT CGCTGAGTAG TGCGCGAGCA AAATTTAAGC 240
 TACAACAAGG CAAGGCTTGA CCGACAATTG CATGAAGAAT CTGCTTAGGG TTAGGCGTTT 300
 TGCGCTGCTT CGCGATGTAC GGGCCAGATA TACGCGTTGA CATTGATTAT TGAAGTATTA 360
 TTAATAGTAA TCAATTACGG GGTCAATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC 420
 40 ATAACCTTACG GTAAATGGCC CGCCTGGCTG ACCGCCAAC GACCCCGGCC CATTGACGTC 480
 AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT 540
 GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC 600
 GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC 660
 CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT 720
 45 GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTC 780
 AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGACTT 840
 TCCAAATGT CGTAACAACT CGCCCCATT GACGCAAATG GGCGGTAGGC GTGTACGGTG 900
 GGAGGTCTAT ATAAGCAGAG CTCTCTGGCT AACTAGAGAA CCCACTGCTT ACTGGCTTAT 960
 CGAAATTAAT ACGACTCACT ATAGGGAGAC CCAAGCTTGG TACCAATTTA AATTGATATC 1020
 50 TCCTTAGGTC TCGAGCACCA TGAAGTTGCC TGTTAGGCTG TTGGTGCTGA TGTCTGGAT 1080
 TCCTGCTTCC AGCAGTGATG TTGTCTGAC CCAACCCCA CTGTCCAGTC CTGTACGCT 1140
 TGGACAACCT GCGTCCATCT CTTGCAGATC TAGTCAGATC ATTGTACATA ATAATGGCAA 1200
 CACCTATCTG GAATGGTACC AGCAGAGACC AGGGCAGTCT CCACGGCTCC TGATCTACAA 1260
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 55 TTTCACACTC AAGATCAGCA GAGTGGAGGC TGAGGATGTG GGAGTTTACT ACTGCTTCCA 1380
 GGGTTCACAT GTTCCATTCA CGTTCGGCCA AGGGACAAAG TTGGAAATCA AACGTAAGTC 1440
 TCGAGTCTCT AGATAACCGG TCAATCGATT GGAATTCTAA ACTCTGAGGG GGTCCGATGA 1500
 CGTGGCCATT CTTTGCCTAA AGCATTGAGT TTAGTGCAAG GTCAGAAAAG CATGCAAAGC 1560
 CCTCAGAATG GCTGCAAGA GCTCCAACAA AACAATTAG AACTTTATTA AGGAATAGGG 1620

	GGAAGCTAGG	AAGAACTCA	AAACATCAAG	ATTTTAAATA	CGCTTCTTGG	TCTCCTTGCT	1680
	ATAATTATCT	GGGATAAGCA	TGCTGTTTTC	TGTCTGTCCC	TAACATGCCC	TTATCGGCAA	1740
	ACAACACACC	CAAGGGCAGA	ACTTTGTAC	TTAAACACCA	TCCTGTTTGC	TTCTTTCCTC	1800
5	AGGAACTGTG	GCTGCACCAT	CTGTCTTCAT	CTTCCCGCCA	TCTGATGAGC	AGTTGAAATC	1860
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	GTGGAAGGTG	GATAACGCCC	TCCAATCGGG	TAACCTCCAG	GAGAGTGTCA	CAGAGCAGGA	1980
	GAGCAAGGAC	AGCACCTACA	GCCTCAGCAG	CACCTGACG	CTGAGCAAAG	CAGACTACGA	2040
	GAAACACAAA	GTCTACGCCT	GCGAAGTCAC	CCATCAGGGC	CTGAGCTCGC	CCGTACACAA	2100
10	GAGCTTCAAC	AGGGGAGAGT	GTTAGAGGGA	GAAGTGCCCC	CACCTGCTCC	TCAGTTCCAG	2160
	CCTGACCCCC	TCCCATCCTT	TGGCCTCTGA	CCCTTTTTC	ACAGGGGACC	TACCCCTATT	2220
	GCGGTCTCTC	AGCTCATCTT	TCACCTCACC	CCCCCTCTCC	TCCTTGGCTT	TAATTATGCT	2280
	AATGTTGGAG	GAGAATGAAT	AAATAAAGTG	AATCTTTGCA	CCTGTGGTTT	CTCTCTTTCC	2340
	TCATTTAATA	ATTATTATCT	GTTGTTTTAC	CAACTACTCA	ATTCTCTTA	TAAGGGACTA	2400
	AATATGTAGT	CATCCTAAGG	CACGTAACCA	TTTATAAAAA	TCATCCTTCA	TTCTATTTTA	2460
15	CCCTATCATC	CTCTGCAAGA	CAGTCTCTCC	TCAAACCCAC	AAGCCTTCTG	TCCTCACAGT	2520
	CCCTGGGCC	ATGGTAGGAG	AGACTTGCTT	CCTTGTTTTC	CCCTCCTCAG	CAAGCCCTCA	2580
	TAGTCCTTTT	TAAGGGTGAC	AGGTCCTACA	GTCATATATC	CTTTGATTCA	ATTCCCTGAG	2640
	AATCAACCAA	AGCAAAATTT	TCAAAAGAAG	AAACCTGCTA	TAAAGAGAAT	CATTCAATGC	2700
20	AACATGATAT	AAAATAACAA	CACAATAAAA	GCAATTAAAT	AAACAAACAA	TAGGGAAATG	2760
	TTTAAGTTCA	TCATGGTACT	TAGACTTAAT	GGAATGTCAT	GCCTTATTTA	CATTTTAAAA	2820
	CAGGTACTGA	GGGACTCCTG	TCTGCCAAGG	GCCGTATTGA	GTACTTTCCA	CAACCTAATT	2880
	TAATCCACAC	TATACTGTGA	GATTAAAAAC	ATTCAATAAA	ATGTTGCAAA	GGTTCTATAA	2940
	AGCTGAGAGA	CAAATATATT	CTATAACTCA	GCAATCCAC	TTCTAGATGA	CTGAGTGTCC	3000
25	CCACCCACCA	AAAAACTATG	CAAGAATGTT	CAAAGCAGCT	TTATTTACAA	AAGCCAAAAA	3060
	TTGGAATAG	CCCGATTGTC	CAACAATAGA	ATGAGTTATT	AAACTGTGGT	ATGTTTATAC	3120
	ATTAGAATAC	CCAATGAGGA	GAATTAACAA	GCTACAACCTA	TACCTACTCA	CACAGATGAA	3180
	TCTCATAAAA	ATAATGTTAC	ATAAGAGAAA	CTCAATGCAA	AAGATATGTT	CTGTATGTTT	3240
	TCATCCATAT	AAAGTTCAAA	ACCAGGTAAA	AATAAAGTTA	GAAATTGGA	TGGAAATTAC	3300
30	TCTTAGCTGG	GGGTGGGCGA	GTTAGTGCCT	GGGAGAAGAC	AAGAAGGGGC	TTCTGGGGTC	3360
	TTGGTAATGT	TCTGTTCTCT	GTGTGGGGTT	GTGCAGTTAT	GATCTGTGCA	CTGTCTGTGA	3420
	TACACATTAT	GCTTCAAAAT	AACTTCACAT	AAAGAACATC	TTATACCCAG	TTAATAGATA	3480
	GAAGAGGAAT	AAGTAATAGG	TCAAGACCAA	CGCAGCTGGT	AAGTGGGGGC	CTGGGATCAA	3540
	ATAGCTACCT	GCCTAATCCT	GCCCWCTTGA	GCCCTGAATG	AGTCTGCCTT	CCAGGGCTCA	3600
35	AGGTGCTCAA	CAAAACAACA	GGCTGCTAT	TTTCCTGGCA	TCTGTGCCCT	GTTTGGCTAG	3660
	CTAGGAGCAC	ACATACATAG	AAATTAATG	AAACAGACCT	TCAGCAAGGG	GACAGAGGAC	3720
	AGAATTAAAC	TTGCCCAGAC	ACTGGAACCC	CATGTATGAA	CACTCACATG	TTTGGGAAGG	3780
	GGGAAGGGCA	CATGTAAATG	AGGACTCTTC	CTCATCTAT	GGGGCACTCT	GGCCCTGCC	3840
	CTCTCAGCTA	CTCATCCATC	CAACACACCT	TTCTAAGTAC	CTCTCTCTGC	CTACACTCTG	3900
40	AAGGGGTTCA	GGAGTAACCTA	ACACAGCATC	CCTTCCCTCA	AATGACTGAC	AATCCCTTTG	3960
	TCTGTCTTTG	TTTTCTTTTC	CAGTCAGTAC	TGGGAAAGTG	GGGAAGGACA	GTGATGGAGA	4020
	AACTACATAA	GGGAAGCACCT	TGCCCTTCTG	CCTCTTGAGA	ATGTTGATGA	GTATCAAATC	4080
	TTTCAAACCT	TGGAGGTTTG	AGTAGGGGTG	AGACTCAGTA	ATGTCCCTTC	CAATGACATG	4140
	AACTTGCTCA	CTCATCCCTG	GGGGCCAAAT	TGAACAATCA	AAGGCAGGCA	TAATCCAGTT	4200
45	ATGAATTCTT	GCGGCCGCTT	GCTAGCTTCA	CGTGTGGAT	CCAACCGCGG	AAGGGCCCTA	4260
	TTCTATAGTG	TCACCTAAAT	GCTAGAGCTC	GCTGATCAGC	CTCGACTGTG	CCTTCTAGTT	4320
	GCCAGCCATC	TGTTGTTTGC	CCCTCCCTCG	TGCCCTTCCT	GACCCTGGAA	GGTGCCACTC	4380
	CCACTGTCCT	TTCTTAATAA	AATGAGGAAA	TTGCATCGCA	TTGTCTGAGT	AGGTGTCATT	4440
	CTATTCTGGG	GGGTGGGGTG	GGGCAGGACA	GCAAGGGGGA	GGATTGGGAA	GACAATAGCA	4500
50	GGCATGCTGG	GGATGCGGTG	GGCTCTATGG	CTTCTGAGGC	GGAAAGAACC	AGCTGGGGCT	4560
	CTAGGGGGTA	TCCCCACGCG	CCCTGTAGCG	GCGCATTAAG	CGCGGCGGGT	GTGGTGGTTA	4620
	CGCGCAGCGT	GACCGCTACA	CTTGCCAGCG	CCCTAGCGCC	CGCTCCTTTC	GCTTTCTTCC	4680
	CTTCCTTTCT	CGCCACGTTT	GCCGGGCCTC	TCAAAAAAGG	GAAAAAAGC	ATGCATCTCA	4740
	ATTAGTCAGC	AACCATAGTC	CGCCCCCTAA	CTCCGCCCAT	CCCGCCCCCTA	ACTCCGCCCA	4800
55	GTTCGCCCA	TTCTCCGCCC	CATGGCTGAC	TAATTTTTTT	TATTTATGCA	GAGGCCGAGG	4860
	CCGCCTCGCG	CTCTGAGCTA	TTCCAGAAGT	AGTGAGGAGG	CTTTTTTGGA	GGCCTAGGCT	4920
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	AGCGTGAAGG	CTGGTAGGAT	TTTATCCCCG	CTGCCATCAT	GGTTGACCA	TGGAAGTGA	5040
	TCGTGCGCGT	GTCCCAAAAT	ATGGGGATTG	GCAAGAACGG	AGACCTACCC	TGGCCTCCGC	5100
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	ATTTTCTTGC	CAAAAGTTTG	GATGATGCCT	TAAGACTTAT	TGAACAACCG	GAATTGGCAA	5340
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5	AACCAGGCCA	CCTTAGACTC	TTTGTGACAA	GGATCATGCA	GGAATTTGAA	AGTGACACGT	5460
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	AGAAATGCCA	TCTAGTGATG	ATGAGGCTAC	TGCTGACTCT	CAACATTCTA	CTCCTCCAAA	5940
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55	ATAATTCTCT	TACTGTCTATG	CCATCCGTAA	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	8460
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AAGTGCCACC	TGACGTC					8897

What is claimed is:

1. A method for inhibiting immunoglobulin-induced toxicity resulting from
5 immunoglobulin immunotherapy in a subject comprising administering an
immunoglobulin molecule to the subject, the immunoglobulin molecule
having a variable region and a constant region, the immunoglobulin molecule
being modified prior to administration by structurally altering multiple
toxicity associated domains in the constant region so that immunoglobulin-
10 induced toxicity is inhibited.
2. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunoglobulin immunotherapy in a subject comprising administering a
structurally altered antibody to the subject, the structurally altered antibody
15 comprising a variable region and a constant region, multiple toxicity
associated domains in the constant region being modified so as to render the
constant region unable to mediate an ADCC response or activate
complement thereby inhibiting immunoglobulin-induced toxicity resulting
from immunotherapy.
- 20 3. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein having multiple structurally altered toxicity
associated domains in the constant region.
- 25 4. A method for inhibiting immunoglobulin-induced toxicity resulting from
immunotherapy in a subject comprising administering an Ig fusion protein to
the subject, the Ig fusion protein comprising a modified constant region, the

modification being a structural alteration in multiple toxicity associated regions within the CH₂ domain.

5. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an immunoglobulin which recognizes and binds a target, the target being associated with the disease;
- (b) mutating the immunoglobulin so selected by structurally altering multiple toxicity associated domains in the constant region of the immunoglobulin thereby creating a structurally altered immunoglobulin;
- (c) administering the structurally altered immunoglobulin of step (b) to the subject under conditions so that the structurally altered immunoglobulin recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the constant region thereby preventing immunoglobulin-induced toxicity in the subject.
6. A method for preventing immunoglobulin-induced toxicity resulting from immunotherapy for a disease in a subject comprising:
- (a) selecting an Ig fusion protein which recognizes and binds a target, the target being associated with the disease;
- (b) structurally altering multiple toxicity associated domains in the CH₂ domain of the constant region of the Ig protein so selected;

- 5 (c) administering the structurally altered Ig fusion protein of step (b) to the subject under conditions so that the structurally altered Ig fusion protein recognizes and binds the target thereby alleviating symptoms associated with the disease, the structural alteration of the CH₂ domain thereby preventing immunoglobulin-induced toxicity in the subject.
- 10 7. The method of claim 1, 2, 3, 4, 5, or 6, wherein the portion of the constant region is the CH₂ domain.
8. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgG.
9. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgM.
- 15 10. The method of claim 1 or 5, wherein the immunoglobulin molecule is IgA.
11. The method of claim 2, wherein the antibody recognizes and binds Le^y.
- 20 12. The method of claim 2, wherein the antibody recognizes and binds to Le^x.
13. The method of claim 2, wherein the antibody is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
- 25 14. The method of claim 2, wherein the antibody is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.

15. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds Le^y.
16. The method of claim 1 or 5, wherein the immunoglobulin recognizes and binds to Le^x.
17. The method of claim 1 or 5, wherein the immunoglobulin is a monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
18. The method of claim 1 or 5, wherein the immunoglobulin is a chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
19. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds Le^y.
20. The method of claim 3, 4, or 6, wherein the Ig fusion protein recognizes and binds to Le^x.
21. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of monoclonal antibody BR96 produced by the hybridoma having the identifying characteristics of HB 10036 as deposited with the ATCC.
22. The method of claim 3, 4, or 6, wherein the Ig fusion protein is a derivative of chimeric antibody ChiBR96 produced by the hybridoma having the identifying characteristics of HB 10460 as deposited with the ATCC.
23. A pharmaceutical composition comprising a pharmaceutically effective

amount of a structurally altered immunoglobulin, and an acceptable carrier, the structurally altered immunoglobulin (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

5 24. A pharmaceutical composition comprising a pharmaceutically effective amount of structurally altered Ig fusion protein, and an acceptable carrier, the structurally altered Ig fusion protein (1) recognizes and binds a target, the target is associated with cancer and (2) has an inactivated CH₂ domain.

10 25. A method of treating carcinomas in vivo comprising administering to a subject a pharmaceutically effective amount of the composition of claim 23 or 24.

15 26. The method of claim 30, wherein the structurally altered immunoglobulin in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

20 27. The method of claim 24, wherein the Ig fusion protein in the composition is labeled so as to directly or indirectly produce a detectable signal with a compound selected from the group consisting of a radiolabel, an enzyme, a chromophore, a chemiluminescer, and a fluorescer.

25 28. The method of claim 2 or 5, wherein the antibody is conjugated to a cytotoxic agent.

29. The method of claim 1, wherein the immunoglobulin is conjugated to a cytotoxic agent.

30. The method of claim 3, 4 or 6, wherein the Ig fusion protein is conjugated to a cytotoxic agent.
31. The method of claim 28, 29, or 31, wherein the cytotoxic agent is selected from the group consisting of antimetabolites, alkylating agents, anthracyclines, antibiotics, anti-mitotic agents, and chemotherapeutic agents.
32. A method for treating a subject suffering from a cancer, the cancer being characterized as a group of cells having a tumor associated antigen on the cell surface, which method comprises administering to the subject a cancer killing amount of the composition of claim 23 or 24 joined to a cytotoxic agent under conditions which permit the molecule so joined to bind the tumor associated antigen on the cell surface so as to kill the cells so bound thereby curing the subject.
33. A pharmaceutical composition comprising a pharmaceutically effective amount of a structurally altered BR96 antibody, the structurally altered antibody having an inactivated CH₂ domain.
34. A method for treating a subject suffering from a proliferative type disease characterized by cells having a BR96 antigen on the cell surface which comprises administering to the subject an effective amount of the composition of claim 33 joined to doxorubicin such that the immunoconjugate binds the BR96 antigen and kills said cells thereby treating the subject.
35. A method for inhibiting BR96 (ATCC: HB10036) induced toxicity resulting from immunoglobulin immunotherapy in a subject comprising administering

BR96 to the subject, the BR96 molecule being modified prior to administration, the modification comprising the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated toxicity is inhibited.

36. A method for preventing BR96 (ATCC: HB10036) induced toxicity resulting from immunotherapy for cancer in a subject comprising:
- (a) mutating the BR96 polypeptide by the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 310-331 and the deletion or substitution of at least one amino acid residue in the toxicity associated domain localized to amino acids 231-238 so that complement and Fc receptor mediated immunoglobulin-induced toxicity is inhibited in the altered BR96 polypeptide; and
- (b) administering the structurally altered BR96 polypeptide of step (a) to the subject under conditions so that the peptide recognizes and binds cancer associated Le^y antigens, thereby alleviating symptoms associated with the cancer, the structural alteration of the toxicity associated domains thereby preventing BR96 toxicity in the subject.
37. A chimeric BR96 antibody having a structurally altered constant region having the CH1 and CH3 domains but not the CH2 domain, the antibody being designated cBR96-A.

38. The chimeric BR96 antibody of claim 37 which is expressed by the plasmid having the sequence shown in SEQ ID NO. 10.
39. A BR96 antibody having humanized variable and constant regions, wherein
5 the constant region has been structurally altered so that the CH1 and CH3 domains are present but the CH2 domain is not, the antibody being designated hBR96-2A.
40. The BR96 antibody of claim 39 which is expressed by the plasmid having
10 the sequence shown in SEQ ID NO. 12.
41. A BR96 antibody designated hBR96-2B having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine and glycine at amino acid position 237 is mutated to alanine.
15
42. A BR96 antibody designated hBR96-2C having a structurally altered constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.
20
43. A BR96 antibody designated hBR96-2D having a structurally altered constant region wherein proline at amino acid position 331 is mutated to alanine.
- 25 44. A BR96 antibody designated hBR96-2E having a structurally altered constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid

position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine.

45. A BR96 antibody designated hBR96-2F having a structurally altered
5 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; and proline at amino acid position 331 is mutated to alanine.
46. A BR96 antibody designated hBR96-2G having a structurally altered
10 constant region wherein glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; and lysine at amino acid position 322 is mutated to serine; and proline at amino acid position 331 is mutated to alanine.
47. A BR96 antibody designated hBR96-2H having a structurally altered
15 constant region wherein leucine at amino acid position 235 is mutated to alanine; glycine at amino acid position 237 is mutated to alanine; glutamic acid at amino acid position 318 is mutated to serine; lysine at amino acid position 320 is mutated to serine; lysine at amino acid position 322 is
20 mutated to serine; and proline at amino acid position 331 is mutated to alanine.
48. A nucleic acid molecule which encodes the BR96 antibody of claim 37, 39, and 41-47.
25
49. A cDNA of claim 48.
50. A plasmid which comprises the nucleic acid molecule of claim 48.

51. A host vector system comprising a plasmid of claim 50 in a suitable host cell.
52. A method for producing a protein comprising growing the host vector system of claim 51 so as to produce the protein in the host and recovering the protein so produced.
- 5

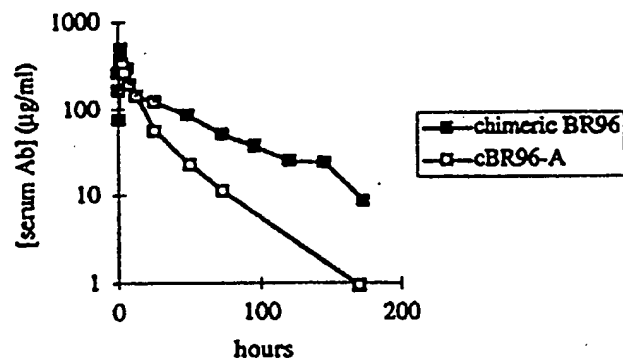


Figure 1. Plasma clearance in high LeY expressing dogs chimeric versus constant region mutant of cBR96-2.

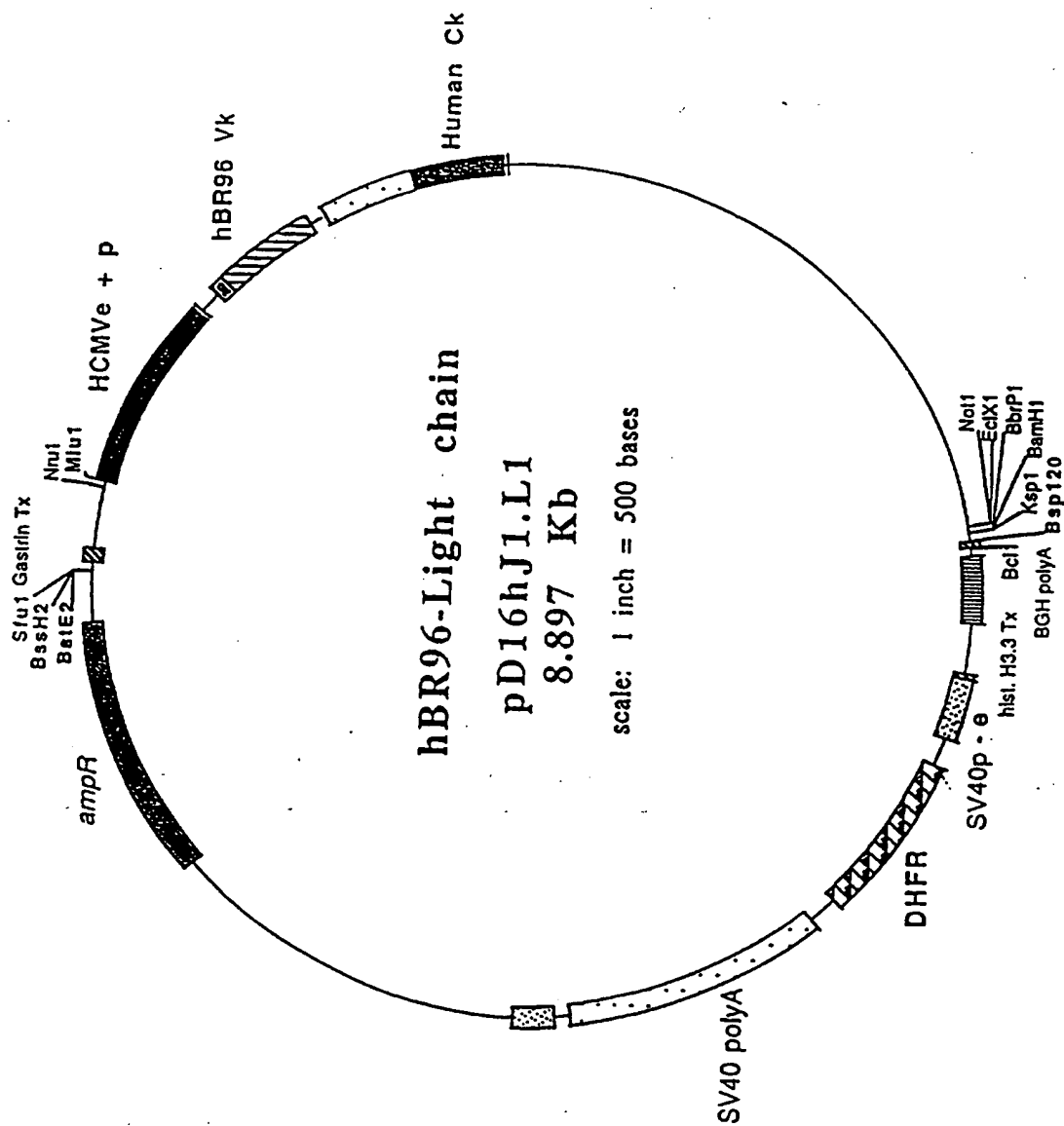
Figure One

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Figure 2



Figure 3



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Figure 4

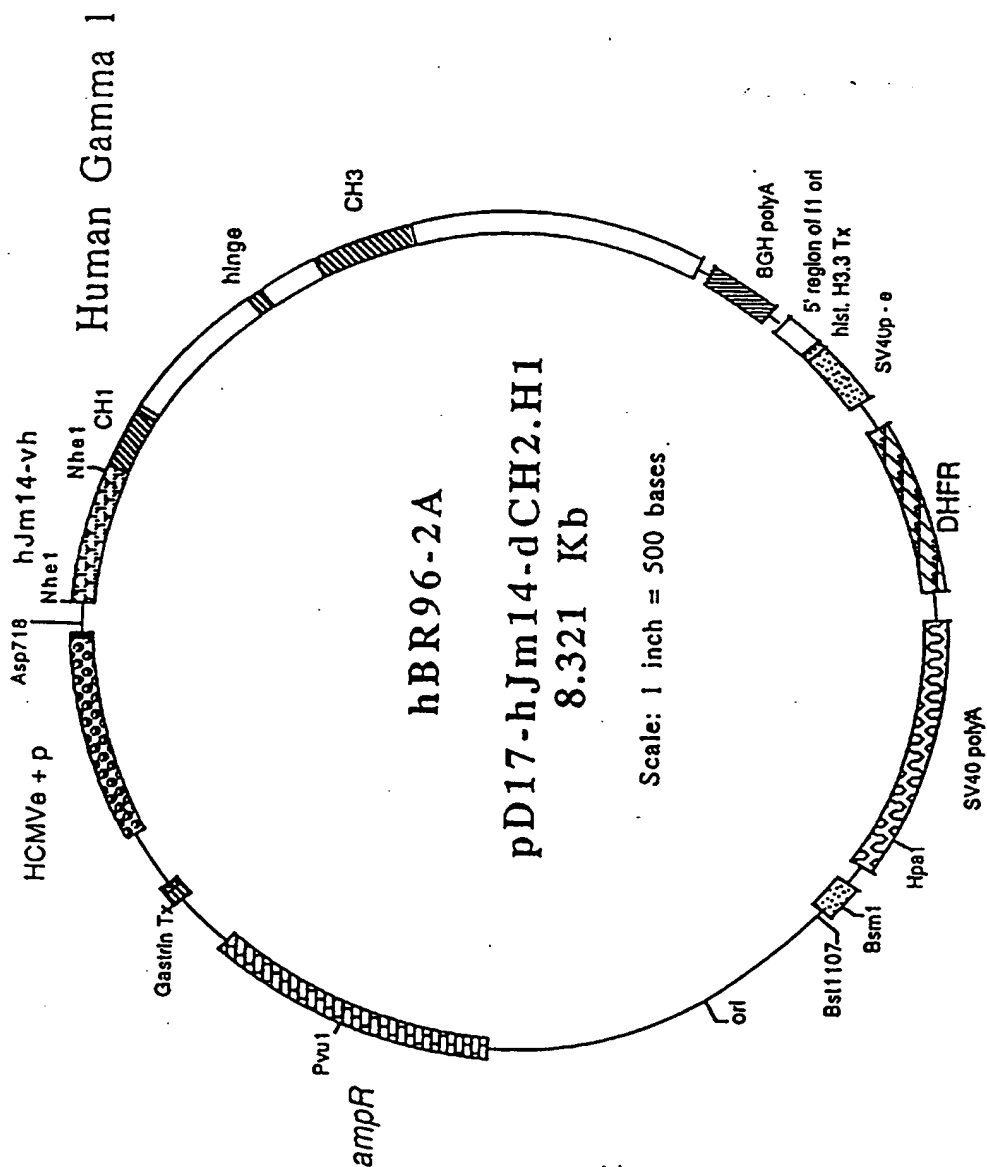


Figure 5

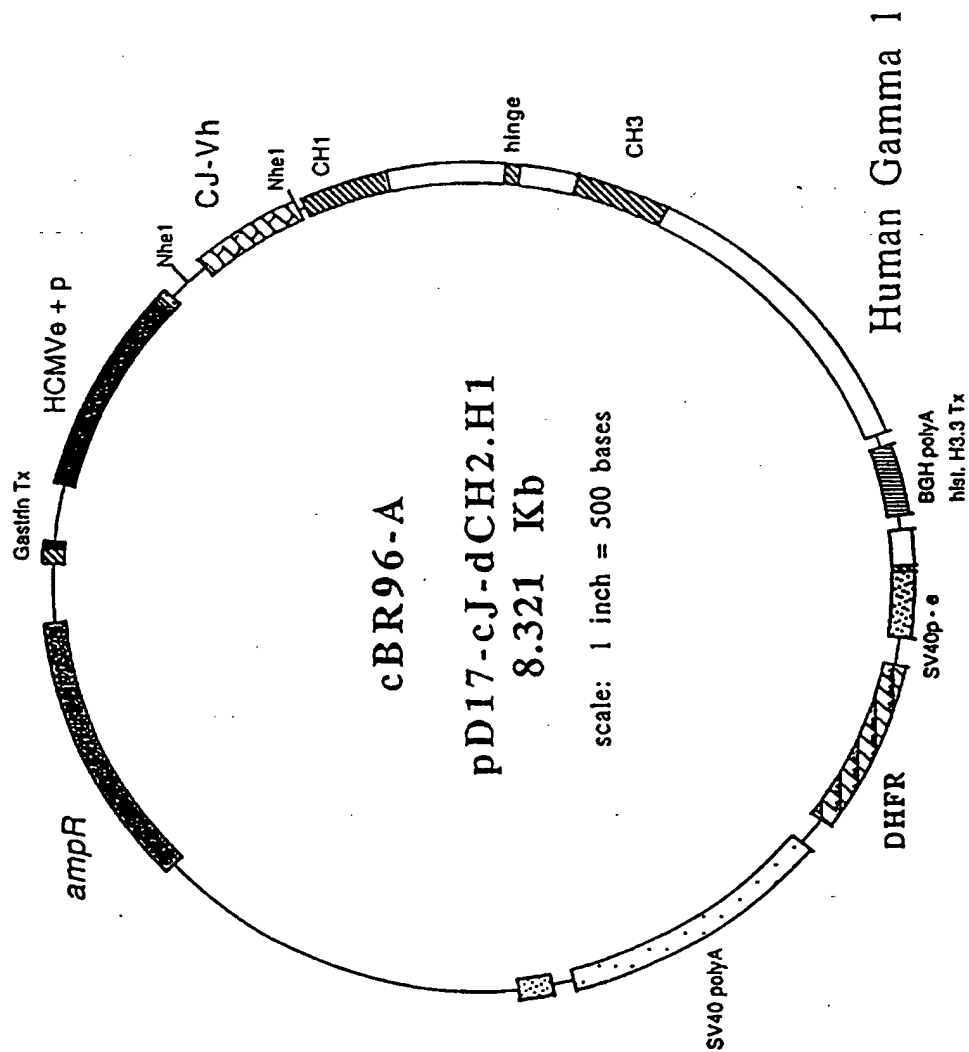
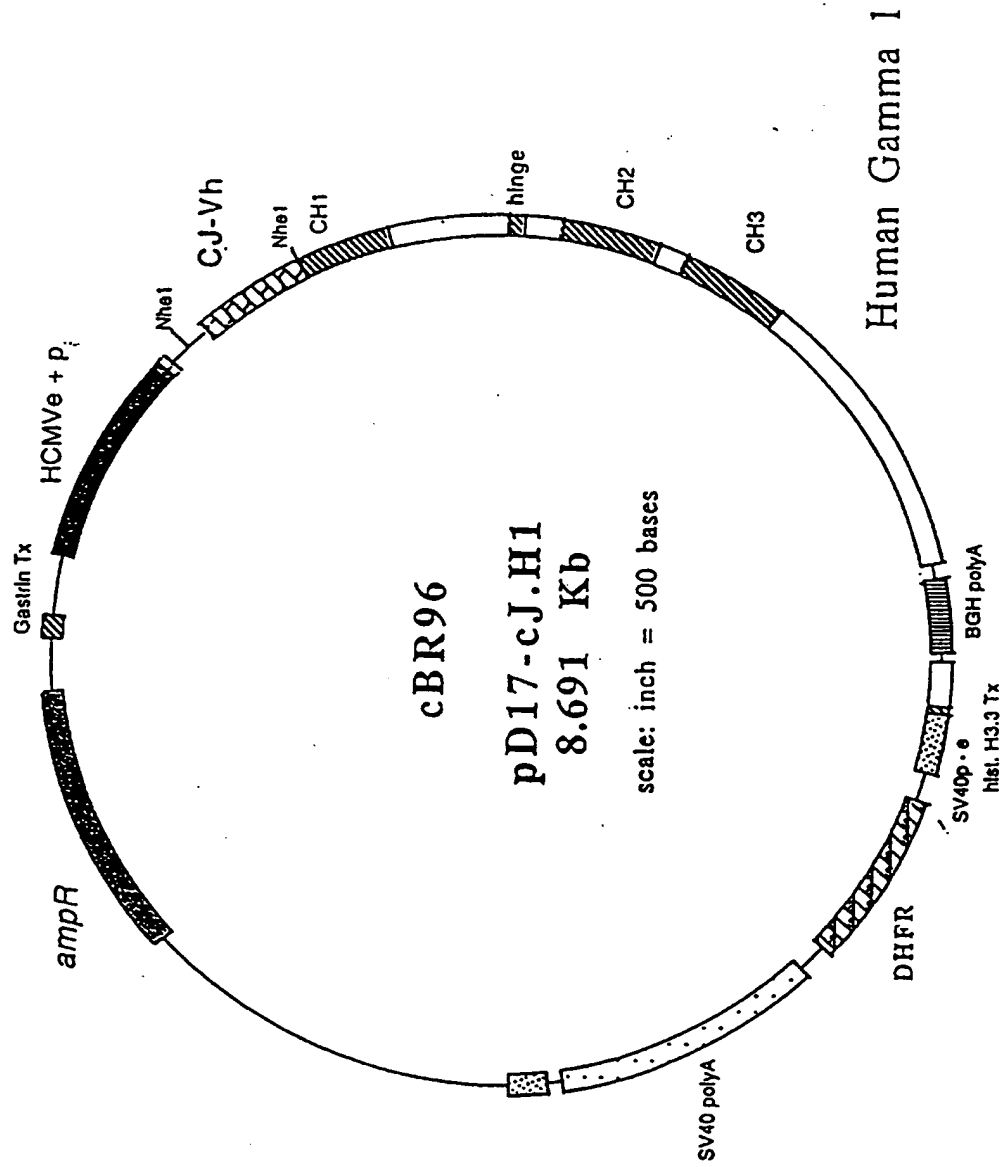
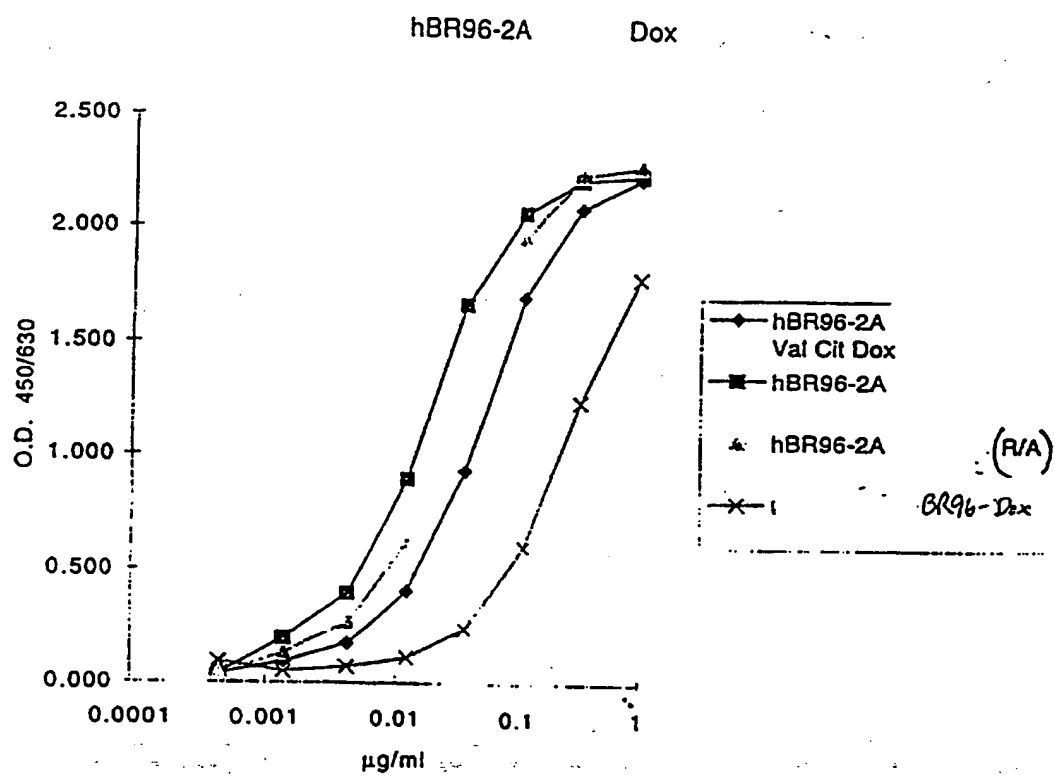


Figure 6



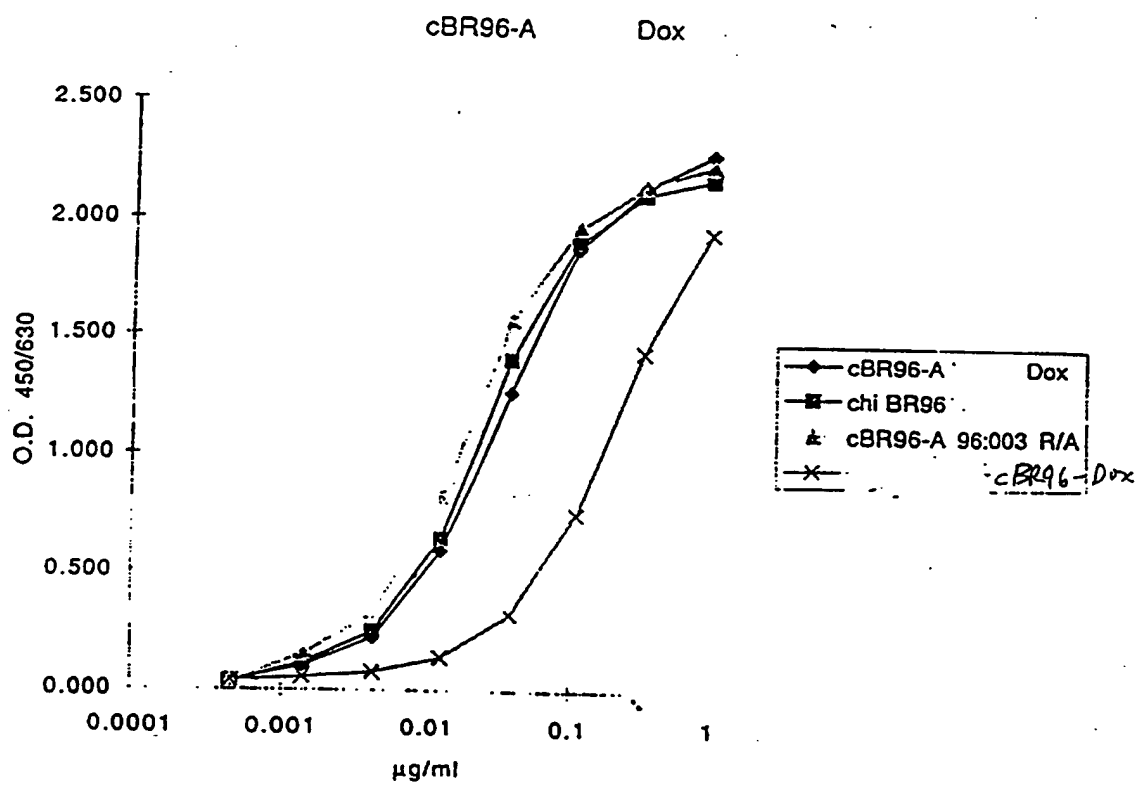
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Figure 7

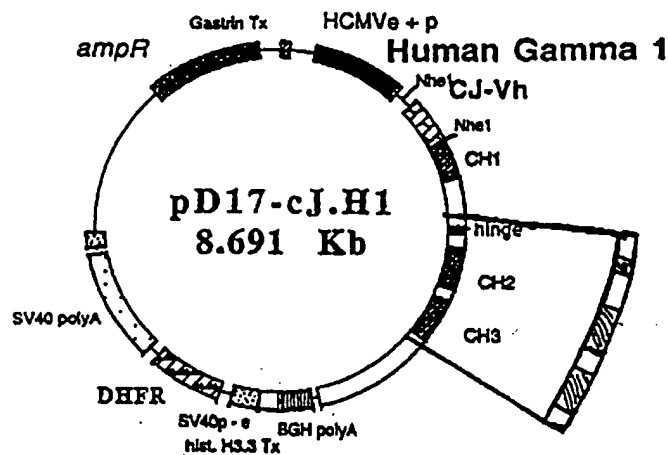


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Figure 8



A- Hinge + CH₁ + CH₃ domains were removed from μ R96 IgG1 construct by E.co NheI restriction digestion.



B. 1 - Hinge + CH₃ domains amplified by PCR from L6 IgG1 construct lacking the CH2 domain.

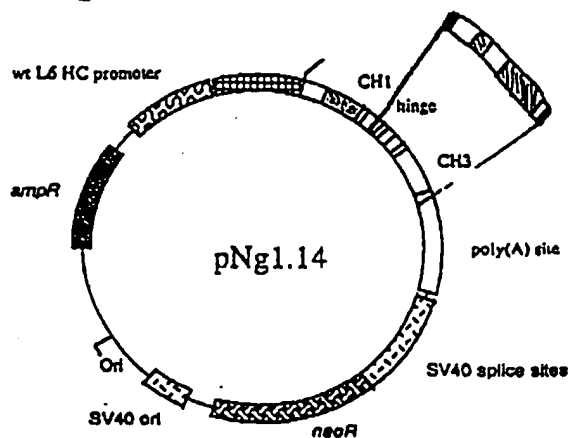


Figure 9

9 - Hinge + CH3 PCR fragment cloned by homologous recombination into E.co47-III site of BR96 IgG1 molecule.

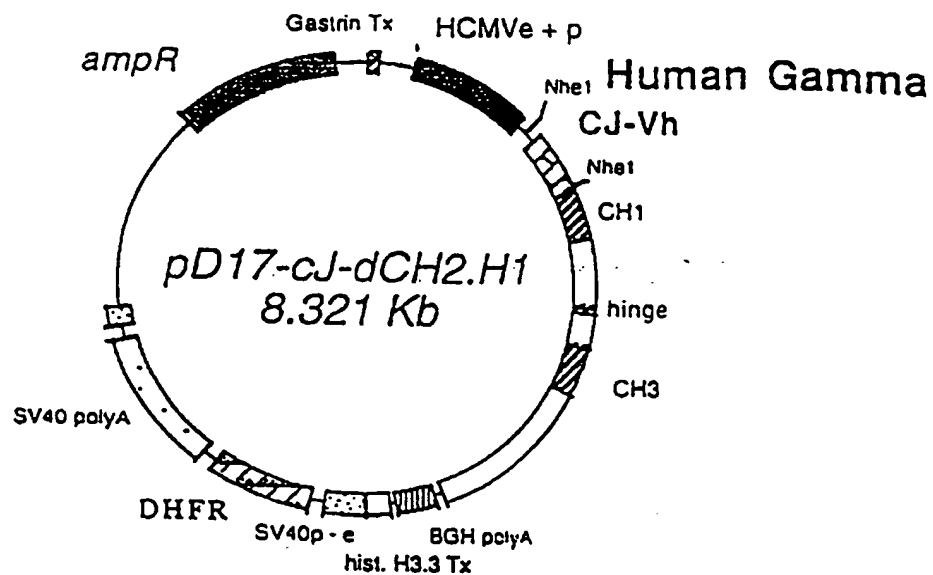
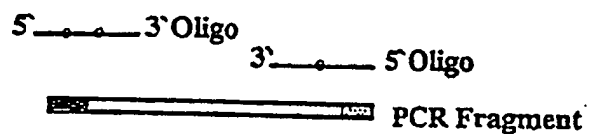


Figure 9

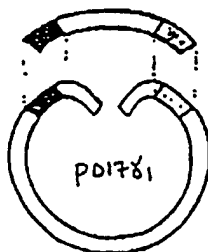
(CONTINUED)

1- Introduction of mutations by site-directed mutagenesis on double-stranded plasmid DNA.

A- Mutations introduced into synthetic oligonucleotides used for the PCR amplification of CH2 domain.



B- Plasmid DNA linearized inside CH2 domain and co-transformed with PCR fragment into competent DH5 α .



C- Cloning mediated by homologous recombination yields transformants harbouring recombinant plasmids.

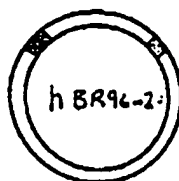
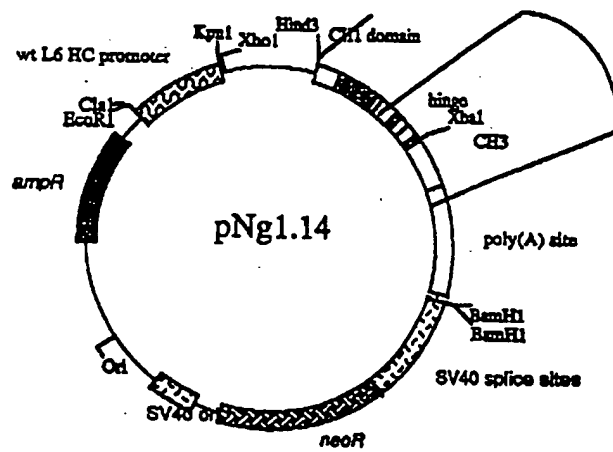


Figure 10

Figure 11



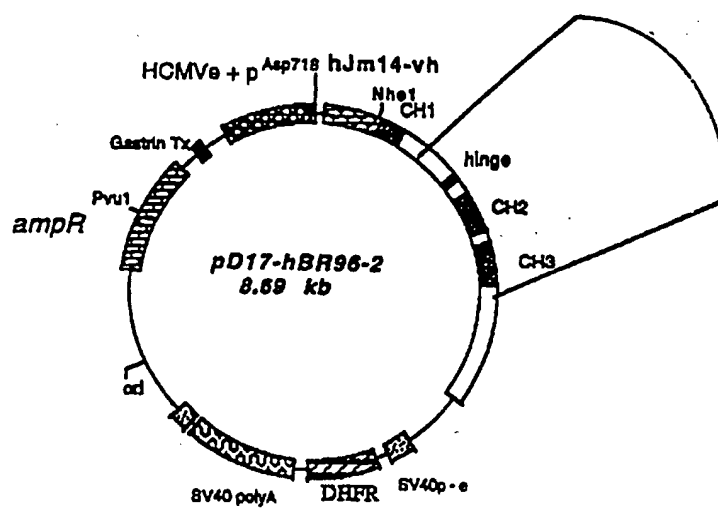


Figure 12

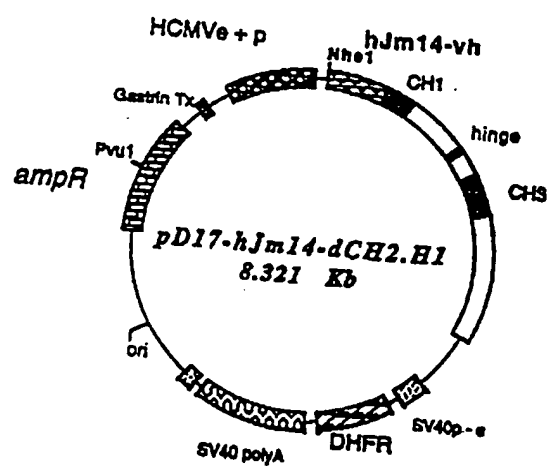


Figure 13

pD17-cJ-dCH2.H1

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 TCTTAGACGA ATCCCATCC GCANACGCG ACNACGCGT ACATGCCCG TCTATATGCG CAACGTATAC TAATAACTGA TCAATAATAA
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 TAAATGCCAT TTGACGGGTG AACCGTATG TAGTTTACAT AGTATACGCT TCATGCCGGG GATAACTGCA GTTACTTGCCA TTATCCCGGC 720
 640 CCTGOCATTA TCGCCAGTAC ATGACCTTAT GGGACTTTCC TACTTTGGCAG TACATCTACG TATTAGTTCAT CGCTATTACC ATGGTATGC 810
 GGACCGTAAT ACGGTGATG TACTGGATA CCGTGAAGG ATGAAACGTC ATGTAGATGC ATATACAGTA GCGATAATGG TACCACCTAGC
 730 GGTTTGGCA GTACATCAAT GGGCGTGGAT AGCGTTTGA CTCACGGGA TTTCCAGTC TCCACCCCAT TGACGTCAAT GGAAGTTTGT 800
 CCAAAACCGT CATGTAGTTA CCGGCACCTA TCGCCAAAT GAGTGGCCCT AAAGTTTCAG AGTGGGTTA ACTGCATTA CCTCAAAACA
 820 TTGCGACCA AATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGC CCATTGACG AATGGCGG TAGGGGTGA CCGTGGAGG 900
 AACCCTGGT TTAGTTGCC CTGAAAGTT TTACAGCAT TTACAGCGG GGTAACTGCG GTTACCCGC ATCCGACAT GCCACCTCC

Figure 14

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910 TCTATATAAG CAGAGCTCTC TGGCTAACTA GAGAAACCCAC TGCTTACTGG CTTATCGAAA TTAATACGAC TCACTATAGG GAGACCCAG 980
AGATATATTC GTCTCGAGAG ACCGATGTAT CTCCTTGGGTG ACGAATGACC GAATAGCTTT AATTATGCTG AGTATATACC CTCTGGGTTC
1000 CTTGGTACCA ATTAAATTG ATATCTCCTT AGGTCTCGAG TCTCTAGATA ACCGGTCAAT CGATTGGAAT TCTTGGGCC GCTTGGTAGC 1080
GAACCATGGT TAAATTTAAC TATAGAGAA TCCAGAGCTC AGAATCTAT TGGCCAGTTA GCTAACTTA AGAACGCCGG CGAACGATCG
1090 CACCATGGAG TTGTGGTTAA GCTTGGTCTT TCCCTGTCCT TCTTTTAAA GGTGTCCAGT GTGAAGTGA TCTGTGGAG TCTGGGGAG 1160
GTGTACCTC AACACCAATT CGAACCCAGGA AGGAACAGGA ACMAAATTTT CCACAGGTCA CACTTCACCT AGACCACCTC AGACCCCTC
1180 GCTTAGTCA GCTTGGAGG TCCCTGAAAG TCTCTGTGT AACCTCTGGA TTCACCTTCA GTGACTATTA CATCTATTGG GTTGGCCAGA 1260
CGAATCAGT CGGACCTCCC AGGACTTTC AGAGGACACA TTGGAGACCT AAGTGAAAGT CACTGATAT GTACATNACC CAAGCGTCT
1270 CTCACAGAA GAGGCTGGAG TGGGTCCAT ACATTAGTCA AGGTGGTGT ATAACCGACT ATCCAGACAC TCTAAAGGT CGATTCAACA 1340
GAGGTCTCT CTCGACCTC ACCGACGTA TGTAAATCAGT TCCACCACTA TATTGGCTGA TAGGTCTGTG ACATTTCCTA GCTAAAGTGT
1360 TCTCCAGAA CAATGCCAG AACACCTGT ACCTGCANAAT GAGCCGTCTG AAGTCTGAGG ACACAGCCAT GTATTACTGT GCAAGAGGCC 1440
AGAGTCTCT GTTACGGTTC TTGTGGACA TGGACCTTAA CTCGGCAGAC TTCAGACTCC TGTGTGGTA CATAATGACA CATTCTCCGG
1450 TGGACGACG GGCCTGTGT GCTTACTGG GCCAAGGGAC TCTGTCTAG GTCTCTGTAG CTAGCACCAA GGGCCCATCG GTCTTCCCTC 1520
ACCTGCTGCC CCGAACCAA CGAATGACCC CGGTTCCTC AGACCAGTGC CAGAGACATC GATCGTGT CCGGGTAGC CAGAAGGGG
1540 TGGCACCTC CTCACAGAG ACCTCTGGG GCACAGGGC CTTGGGTGC CTTGGTCAAG ACTACTTCC CGAACCGGTG ACGGTCTGT 1600
ACCGTGGAG GAGTTCTG TGGAGACCC CGTGTGCGG GGACCCGAGG GACCAGTTC TGAATGAGG GCTTGGCCAC TGCCACAGCA 1680
1630 GGAATCAGG CCGCTGACC AGCGGCTGC ACACCTTCCC GGCTGTCTA CAGTCTCAG GACTCTACTC CCTCAGCAGC GTGGTCAACG 1760
CCTTGAGTCC GCGGACTG TCGCCGACG TGTGGAAGG CCGACAGATC GTGAGAGTCT CAGAGTGTG GAGTGTGTG CACCAGTGGC 1840
1720 TGCCCTCCAG CAGCTTGGC ACCCAGACCT ACATCTGAA CGTGAATCAC AAGCCAGCA ACACCAAGT GGACAAGAA GTTGGTGA 1920
ACGGAGGTG GTCAACCCG TGGGTCTGGA TGTAGACCTT GCACCTAGT TCGGGTCTG TGTGTCCA CCTGTCTTT CAACCACTCT

Figure 14
(continued)

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1810	1820	1830	1840	1850	1860	1870	1880	1890
GGCAGACACA	GGGAGGAGG	GTGTCTCTG	GAAGCCAGG	TCAGCGTCC	TGCTTGACG	CATCCGGCT	ATGACGCCC	ACTCCAGGG
CCGGTCGTGT	CCCTCCCTCC	CACAGAGAC	CTTCGGTCC	AGTCGGAG	ACGGACCTG	GTAGGCGGA	TACGTCGGG	TCAGGTCCCG
1900	1910	1920	1930	1940	1950	1960	1970	1980
AGCAGGCGAG	GCCCGCTCTG	CCCTCTTCAAC	CGGAGGCTC	TGCGCGCCC	ACTCATGCTC	AGGGAGAGG	TCTTCTGGCT	TTTTCCTCCAG
TGTTTCCGTC	CGGGGACAG	GGAGAGTGG	GCCTCCGGAG	ACGGCGGGG	TGAGTAGCAG	TCCCTCTCCC	AGAAGACCGA	AAAGGGGTC
1990	2000	2010	2020	2030	2040	2050	2060	2070
GCTCTGGGCA	GGCAGAGCT	AGGTGCCCT	AACCCAGGC	CTGCACACA	AGGGGCGAGT	GCTGGGCTCA	GACCTGCCAA	GAGCCATATC
CGAGACCCGT	CCGTGTCCGA	TCCACGGGA	TTGGGTCCG	GACGTGTGT	TCCCCGTCCA	CGACCCGAGT	CTGGACGGTT	CTCGGTATAG
2080	2090	2100	2110	2120	2130	2140	2150	2160
CGGGAGGACC	CTGCCCTGA	CCTAAGCCCA	CCCCAAGGC	CAAACTCTCC	ACTCCCTCAG	CTCGGACACC	TTCTCTCCTC	CCAGATTCCA
CCCCCTCCTG	GACGGGACT	GGATTCCGGT	GGGGTTTCCG	GTTCGAGAG	TGAGGGAGTC	GAGCCTGTGG	AAGAGAGGAG	GGTCTAAGGT
2170	2180	2190	2200	2210	2220	2230	2240	2250
GTAACCTCCA	ATCTTCTCTC	TGCAGAGCC	AAATCTTGT	ACAAACTCA	CACATGCCCA	CCGTGCCCG	GTAAGCCAGC	CCAGGCCCTG
CATTGAGGGT	TAGAAGAGAG	ACGTCTCGGG	TTTAGAACAC	TGTTTGTAGT	GTGTACGGGT	GGCACGGTC	CATTCCGGTC	GGTCCGGAGC
2260	2270	2280	2290	2300	2310	2320	2330	2340
CCCTCCAGCT	CAAGGGCGGA	CAGGTGCCCT	AGAGTAGCCT	GCATCCAGGG	ACACACCCAG	TGGGTACCCA	CATGTCCCGA	GGCACATGGA
GGGAGGTGGA	GTTCCGCCCT	GTCCACGGGA	TCTCATCGGA	CGTAGGTCCC	TGTGTGTGTC	ACCCATGGTT	GTACAGGCCCT	CGGTGTACCT
2350	2360	2370	2380	2390	2400	2410	2420	2430
CAGAGGCGG	CTCGGCCAC	CCCTCTGCCCT	GAGAGTGACC	GCTGTACCAA	CCCTGTCTCC	TACAGGGCAG	CCCCGAGAAC	CACAGGTGTA
GTCTCCGGCC	GAGCCGGGTG	GGAGACGGGA	CTCTCACTGG	CGACATGGTT	GGAGACAGGG	ATGTCCGCTC	GGGCTCTTG	GTGTCCACAT
2440	2450	2460	2470	2480	2490	2500	2510	2520
CACCCCTGCC	CCATCCCGGG	ATGAGCTGAC	CAAGRACCAG	GTCAAGCTGA	CCCTGCCCTG	CAAAGGCTTC	TATCCAGCG	ACATCGCCGT
GTGGAGCGG	GGTAGGGCCC	TACTCGACTG	GTCTTTGGTC	CAGTCGBACT	GGACGGACCA	GTTCGGAG	ATAGGTCGC	TGTAGCGGCA
2530	2540	2550	2560	2570	2580	2590	2600	2610
GGAGTGGGAG	AGCAATGGGC	AGCCGGAGAA	CAACTACAA	ACCAGCCTC	CCGTGCTGGA	CTCCGAGCGC	TCCCTTCTTC	TCTACAGCAA
CCTCACCTC	TGTTTACCG	TGCGCCCTT	GTGATGTTT	TGGTCCGGAG	GGACGACCT	GAGGCTCCCG	AGGAGAAAG	AGATGTCTT
2620	2630	2640	2650	2660	2670	2680	2690	2700
GCTCACCTG	GACAGAGCA	GGTGGCAGCA	GGGGAAGTC	TTCTCATGCT	CCGTGATGCA	TGAGGCTCTG	CACAACTACT	ACAGCCAGAA
CGAGTGGCAC	CTGTCTCTGT	CCACCGTCTG	CCCCCTTCCAG	ANAGATACGA	GGCACTACCT	ACTCCGAGAC	GTGTGTGGA	TGTCCGTCTT

Figure 14
(continued)

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2710 GAGCTCTCC CTGCTCTCCG GTAATGAGT GCGAGCGCG 2740 GCGAGCGCG GCGAGCGCG 2750 GCGAGCGCG 2760 GCGAGCGCG 2770 GCGAGCGCG 2780 GCGAGCGCG 2790 GCGAGCGCG
 CTGGAGAGG GACAGAGGCC CATTTACTCA CGCTGCCGCG CGTTCCGGGG CGAGGCGCC GAGAGCGCA GCGTGTCTCT ACGAACCGTG
 2800 GTACCCCTG TACATACTTC CCGGCGCGCC AGCATGGA A TAAAGCATC AGCGTCCCG TGGGCCCTG CGAGACTGTG ATGTTCTTT 2880
 CATGGGGAC ATGTATGAAG GCGCCGCGG TCGTACCTTT ATTTCTGGG TCGGAGCGG ACCCGGGGAC GCTCTGACAC TACCAAGAAA
 2890 CCACGGGTCA GCGCGAGTCT GAGGCTGAG TGGCATGAG GAGGCGAGC GGGTCCCACT GTCCCGACAC TGGCCCAAGC TGTCCAGGTG
 GGTGCCAGT CCGGCTCAGA CTCGCGACTC ACCGTACTCC CTCGCTCTCG CCCAGGGTGA CAGGGGTGTG ACCGGGTCCG ACACGTCCAC 2970
 2980 TCCCTGGCC CCTTAGGGT GGGCTCAGC AGGGCTGCC 3010 3020 3030 3040 3050 3060
 ACGGACCGG GCGATCCAC CCGGATCCG TCCCGGAGC GAGCGGTCC ACCCTTAA CGGTGCGAC GGGAGGAGG TCGTCTGGA
 3070 GCGTGGCT GCGCCAGCG AGCCCTAGG AGCCCTGG GACAGACACA CAGCCCTGC CTCGTGTAGA GACTGTCTG TTCTGTGAGC 3150
 CCGGACCGA CCGGTGCC TTCCGGATCC TCGGGGACC CTGTCTGT GTCCGGAGC GAGCATCTCT CTGACAGGAC AAGACACTCG
 3160 GCGCTGCT TCCCGACCTC CATGCCCACT CCGGGGCGAT GCTAGTCCAT GTGCTAGG ACAGGCCCTG CCTACCCAT CTACCCCGAC 3240
 CCGGACAGG AGGCTGGAG GTACGGGTGA GCGCCCTAC GCGCCCTAC CACGATCC CTCGCGGAG TGTCCGGAG GAGTGGTA GATGGGGTG
 3250 GCGACTAAC CCTGCTGC CTGCCAGC GACGGGTGG AGCGTGGCG TACCCCTGT TTGGCTAGG CCGCTGTAG TGGAGCGCG GACACCTCC 3310
 CCGTGAATTG GACCGACCG GACGGGTGG AGCGTGGCG TACCCCTGT TTGGCTAGG CCGCTGTAG TGGAGCGCG GACACCTCC 3320
 3340 GACTGTGCA GATGCCCA CACACACTCA GCGCAGACC GCGCAGACC CCGCGACTG AGGTGGCGG GCGCAGCGC CACACACAC 3420
 CTGACACGT CTACGGGT GTGTGTGAGT CCGGTCTGG CAAGTGTGT GCGGCTGAC TCCAAACCGC CCGTGTCCG GTGGTGTG
 3430 ACACGTGAC GCGTCACACA CCGAGCCTCA CCGGGGCGA CTGACAGCA CCCAGACCG AGCAAGTCC TCGCACACGT GAACACTCT 3510
 TGTGACGTG CCGAGTGTGT GCGTGGAGT GCGCCCGCTT GAGTGTCTG GGTCTGTC TCGTTCCAG AGGTGTGCA CTGTGAGGA
 3520 CCGACACAG CCGCCAGCG CACCTCAG CCGCAGGCC CTCGGGAGC TTCTCCACAT TCTCCACTG CCGTGTGAGC TCGACACAC 3600
 GCGTGTGTC GCGGTGCTC GCGGTGCGC GTGGAGTTC GGTGCTCG AGAGCGGTG AAGAGGTGA CCGTGTGAGC AGTCTGTG

Figure 14
(continued)

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3610 3620 3630 3640 3650 3660 3670 3680 3690
CCAGCCCTCC TCTCACAAGG GTGCCCCCTGC AGCGCCACACA CACACACAGG GGATCACACA CCACGTACAG TCCCTGGCCC TGCCCCACTT
GGTCGGAGG AGAGTGTTC CACGGGACG TCGGCGGTGT GTGTGTGTCC CCTAGTGTGT GGTGACGTGC AGGACCGGG ACCGGGTGAA

3700 3710 3720 3730 3740 3750 3760 3770 3780
CCCACTGCTG CCTTCCCTG CAGGACGGAT CAGCTTCGAC GTGCGAGCTG ACACGGAAGA TCAACGGTGC GTAGACAAACA AACGGGAGG GGGCAGCGAA
GGGTACCGC GGGAAAGGAC GTCTGCCCTA GTGCGAGCTG ACACGGAAGA TCAACGGTGC GTAGACAAACA AACGGGAGG GGGCAGCGAA

3790 3800 3810 3820 3830 3840 3850 3860 3870
CCTTGACCT GGAAGGTGC ACTCCCACTG TCCTTTCCTA ATAAATGAG GAAATGCAAT CGCATGTGCT GAGTAGGTGT CATTCCTATT
GGAAGTGGG CCTTCCACGG TGAGGTGAC AGGAAAGAT TATTTTACTC CTTTAACTGA GCGTAACAGA CTCATCCACA GTAAAGATAAG

3880 3890 3900 3910 3920 3930 3940 3950 3960
TGGGGGTGG GGTGGGTCAG GACAGCAAGG GGGAGGATTC GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGCTCT ATGGCTTCTG
ACCCCCCACC CCACCCGTC CTGTGTGTTCC CCTTCTTAC CCTTCTGTTA TCCTCCGTAC GACCCCTAGC CCACCCGAGA TACCGAAGAC

3970 3980 3990 4000 4010 4020 4030 4040 4050
AGCGGGAAG AACCACTG GGTCTTAGGG GGTATCCCA CGGCGCTGT AGCGCGCAT TAAAGCGCGC GGTGTGGTG GTTACGCGCA
TCCGCTTTC TTGGTCGACC CCGAGATCCC CCATAGGGGT CCATAGGGGT GCGCGGACA TCGCCCGCTA ATTCCGCGCG CCCACACAC CAATGCGCT

4060 4070 4080 4090 4100 4110 4120 4130 4140
GGGTACCGC TACACTTGC AGCGCCCTAG CGCGCGCTCC TTTCGGTTTC TTCCCTTCTT TCTCGCCAC GTTCGCGCGG CCTCTCAAAA
CGCACTGGC ATGTGAACG TCOCGGGATC GCGGCGGAGG AAGCGGAAG AAGGAAAGG AAGAGCGGTG CAAGCGGCC GAGAGTGT

4150 4160 4170 4180 4190 4200 4210 4220 4230
AAGGGAATA AAGCATGCAT CTCATTTAGT CAGCAACCAT AGTCCCGCCC CTAACTCCGC CCATCCCGC CCTAACTCCG CCCAGTTCG
TTCCCTTTT TCGTAGCTA GATTTAATCA GTCGTTGTA TCAGGGCGG GATTGAGGG CCGTAGGGC GGTAGAGGC GGTCAAGGC

4240 4250 4260 4270 4280 4290 4300 4310 4320
CCCATCTCC GCGCCATGGC TGACTAATTT TTTTATTTA TGCAGAGGCC GAGCGCCCT CGGCTCTGA GCTATTCCAG AAGTAGTGAG
GGTAAAGG CGGGTACCG ACTGATTA AATAATAT AGTCTCCG CTCCGCGGA GCGGAGACT CGATAAGTC TTCAATCCTC

4330 4340 4350 4360 4370 4380 4390 4400 4410
GAGGCTTTT TGGAGGCTA GGTCTTGA AAAAGCTTGG ACAGCTCAGG GCTCGGATTT CGCGCCAAAC TTGACGCAA TCCTAGCGTG
CTCCGAAA ACCTCCGAT CCGAAACGT TTTTCGAACC TGTGAGTCC CGACCTAA GCGCGGTTG AACTGCGTT AGGATCGCAG

4420 4430 4440 4450 4460 4470 4480 4490 4500
AAGCTGTA GATTTTATC CCGCTGCA TCATGTTGG ACCATTGAAC TGCATCGTG CCGTGTCCA AATATGGG ATTGCAAGA
TTCCGACCAT CCTAATATG GGGGACGGT AGTACCAAGC TGGTAATG TGGTAATG ACGTAGCAG GGCACAGGT TTTATACCC TAACGTTCT

Figure 14
(continued)

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4510 ACCGAGACCT ACCCTGGCCT CCGCTCAGGA ACCAGTTCAAA GACTTCCAA AGAATGACCA CAACTCTTC ACTGGAGGT AACAGAAATC 4590
 TGCCTCTGGA TGGGACCGGA GCGGAGTCTT TGCCTCAAGTT CATGAGGTT TCTTACTGGT GTTGGAGAG TCACCTTCCA TTCTCTCTAG
 4600 TCGTGAATTAT GGGTAGGAAA ACCTGGTTCT CCATTCCTGA GAAGATCGA CCTTTAAAGG ACAGAAATTA TATAGTTCTC AGTAGAGAAC 4680
 ACCACTAATA CCCATCCTTT TGGACCAAGA GGTAAAGGACT CTCTTAGCT GGAATTTCC TGCTTAATT ATATCAAGAG TCATCTCTTG
 4690 TCAAAGAAC ACCACGAGGA GCTCATTTTC TTGCCAAAG TTGGATGAT GCCTTAAGAC TTATTGAACA ACCGGAATTG GCAAGTAAAG 4770
 AGTTTCTTGG TGGTCTCTCT CGAGTAAAG AACGTTTTC AACCTACTA CGGATTTCTG AATAACTTGT TGGCCTTAAC CGTTCATTTC
 4780 TAGACATGGT TTGGATAGTC GGAGGCAGTT CTGTTTACCA GGAAGCAGT AATCAACCAG GCCACCTTAG ACTCTTTGTG ACAAGGATCA 4860
 ATCTGRACCA AACCTATCAG CCTCCGTCAA GACAAATGCT CCTTCGGTAC TTAGTTGGTC CCGTGGAAATC TGAGNAACAC TGTCTCTAGT
 4870 TGCAGGAAT TGAAAGTGAC ACCTTTTTC CAGAAATTGA TTTGGGAAA TATAAACTTC TCCAGAAATA CCCAGCGTC CTCTCTGAGG 4950
 ACGTCTCTAA ACTTTCACG TGCMAAAGG GTCTTTAACT AAACCCCTTT ATATTGAAG AGGTCTTAT GGGTCCGAG GAGAGACTCC
 4960 TCCAGGAGGA AAAAGGCATC AAGTATAAGT TTGAAGTCTA CGAAGAGAA GACTAACAGG AAGATGCTTT CAACTCTCT CTCTCCCTCC 5040
 AGGTCTCTCT TTTTCCGTAG TGCATATTA AACTTCAGT GCTCTTCTTT CTGATTTGCC TTCTACGAAA GTTCAAGAGA CGAGGGGAGG
 5050 TAAAGCTATG CATTTTATA AGACCATGGG ACTTTGCTG GCTTAGATC TCTTTGTGA GGAACCTTAC TTCTGTGGTG TGACATAATT 5130
 ATTTCCGATC GTAAAATAT TCTGTACCC TGAACACGAC CGAAATCTAG AGAAACACTT CCTTGAATG AAGACACCAC ACTGTATTAA
 5140 GGACAACTA CCTACAGAGA TTTAAAGCTC TAAGGTAAT ATAAATTTT TAAGTGTATA ATGTTTAAA CTACTGATTC TAATTTGTTG 5220
 CCTGTGTAT GGAATCTCT AATTTCCGAG ATTCCATTTA TATTTTAAA ATTACATAT TACACAAAT GATGACTAAG ATTAACAAAC
 5230 TGTATTTTAG ATTCCACCT ATGGAACCTGA TGAATGGGAG CAGTGTGGA ATGCCCTTAA TGAGGNAAC CTGTTTGTCT CAGAAGAAAT 5310
 ACATAAATC TAAGTTGA TACCTTGACT ACTTACCCTC GTCCACCT TACGGAATTT ACTCTTTTG GACAAAACGA GTCTCTTTTA
 5320 GCCATCTAGT GATGATGAGG CTACTGCTGA CTCTCAACT TCTACTCTC CAAAAGAA GAGAAAGTA GAAGACCCCA AGGACTTTCC 5400
 CGGTAGATCA CTACTACTCC GATACGACT GAGATTCTA AGATGAGGAG GTTTTCTCTT CTCTTCCAT CTCTGGGT TCCTGAAAGG

Figure 14
(continued)

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5410 TTCAGATTG CTAAGTTTTT TGAGTCATGC TGTGTTTAGT AATAGAACTC TTGCTTGCTT TGCATATTAC ACCACAAAGG AAAAAGCTGC 5490
 AAGTCTTAAC GATTCNAAA ACTCAGTACG ACACAATCA TTAICTTGAG AACGACGAA ACGATAAATG TGGTGTTC TTTTGGACG
 5500 ACTGCTATAC AAGAAATTA TGGAAAATA TTCTGTAAAC TTTATAAGTA GGCATAACAG TTATATCAT AACATACTGT TTTTCTTAC 5580
 TGACGATATG TTCTTTTAAAT ACCTTTTAT AAGACATTTG AATATTATCAT CCGTATTGTC AATATTAGTA TTGTATGACA AAAAGATG
 5590 TCCACACAGG CATAGAGTGT CTGCTATTAA TAACTATGCT CAATAATGT GTACCTTTAG CTTTTTAATT TGTAAAGGGG TTATAAGGA 5670
 AGGTGTGTC GTATCTACA GACGTAAT ATTGATACGA GTTTTAACA CATTGAAATC GAAAAATTA ACATTTCCCC AATTATTCTT
 5680 APATTGATG TATAGTGCCT TGACTAGAGA TCATATCAG CCATACCACA TTTGTAGAGG TTTTACTTGC TTTAAAAAC CTCACACACC 5760
 TATAACTAC ATATCACGA ACTGATCTCT AGTATTAGTC GGTATGCTGT AAACATCTCC AAATGNAAG AAATTTTTC GAGGTGTGG
 5770 TCCCCCTGAA CCTGAACAT AAATGAATG CAATTGTGT TGTAACTTG TTTATTCCAG CTTATATGG TTACAAATA AGCAATAGCA 5850
 AGGGGACTT GGACTTTGTA TTTTACTTAC GTTAACAACA ACAATTGNAC AATAAACGTC GAATATTACC AATGTTTAT TCCTATATGT
 5860 TCACAAATTT CACAAATTA GCATTTTTTT CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT GTCTGGATCG 5940
 AGTGTTTAA GTGTTTATTT CGTAAAAAA GTGACGTAG ATCAACACCA CACAGGTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC
 5950 GCTGGATGAT CTTCCAGCGC GGGATCTCA TGCTGGAGTT CTTCCGCCAC CCCAACTGT TTATTGCAGC TTATATGGT TACAAATAAA 6030
 CGACCTACTA GGAGTGGC CCCTAGAGT ACGACCTCA GAAGCGGTG GGTGTGAACA AATAACGTC AATAATTCCA AATGTTTAT 6120
 GCATAGCAT CACAAATTC ACAATAAAG CATTTTTTC ACTGCATTC AGTTGTGGT TGTCCAACT CATCAATGTA TCTTATCATG
 CGTATATCGTA GTGTTTAAAG TGTATTATTC GTAAAAAAG TGACGTAGA TCACACCAA ACAGGTTTGA CTAGTTACAT AGAATAGTAC
 6130 TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGCGTATC ATGGTCATAG CTGTTTCCCTG TGTGAAATG TTATCCGCTC ACATTTCCAC 6210
 AGACATATGG CAGCTGAGA TCGATCTCGA ACCGCATTAG TACCAGTATC GACAAAGGAC ACATTTTAC AATAGCGAG TGTAAAGGTG
 6220 ACAACATAG AGCCGAGC ATAAAGTGA AAGCCTGGG TGCCATATGA GTGAGCTAAC TCACATTAAT TGGCTTGGC TCACTGGCG 6300
 TGTGTATGC TCGGCTTGC TATTTACAT TTGGAACCC ACGGATTAAT CACTCGATTG AGTGTATTA ACGCAACGG AGTACGGGC

Figure 14
(continued)

6310	6311	6320	6330	6340	6350	6360	6370	6380	6390
CYTTCCAGTC	GGNAACCTG	GGNAACCTG	TCGTGCCAGC	TGCATTAATG	AATCGGCCAA	CGCGCGGGGA	GAGGGCGGTTT	GGCTATTGGG	CGCTCTTCGG
GNAAGGTGAC	CCCTTTGGAC	AGCACGGTGC	ACGTAATTAC	TTAGCCGGTT	CGCGGCCCTT	CGCGGCCCTT	CTCCGCCCAA	CGCATAAACC	CGGAGAAAGC
6400	6410	6420	6430	6440	6450	6460	6470	6480	6490
CCTCCCTCGCT	CACCTGACTG	CTGCGCTCGG	TCGTTCGGCT	GGGCGGAGCG	GTATCAGCTC	ACTCAAAGGC	GGTAATAGCG	TTATCCACAG	TTATCCACAG
GAAGAGCGGA	GTGACTGAGC	GAAGCGAGCC	AGCAAGCCGA	CGCGCGCTGC	CATAGTCCAG	TGAGTTTCCG	CCATTATGCC	AATAGGTGTC	
6580	6590	6600	6610	6620	6630	6640	6650	6660	6670
AATCAGGGGA	TAACGCAGGA	AAGAACATGT	GAGCAAAAGG	CCAGCAAAAG	GGTGGTTTTC	GGTTCCTCTG	GTAAAAAGCG	CGCTTTTTCG	CGCTTTTTCG
TTATGTCCTCT	ATTGCGTCTT	TTCTTGTAACA	CTCGTTTTCG	GGTGGTTTTC	GGTTCCTCTG	CAATTTTTCG	GGCAACGAC	CGCAAAAGG	
6680	6690	6700	6710	6720	6730	6740	6750	6760	6770
ATAGGCTTCG	CCCGCTGAC	GAGCATCAAC	AAATTCGAGC	CTCAAGTTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAAGCGT	TACCAAGCGT
TATCCGAGGC	GGGGGACTG	CTCGTAGTGT	TTTTAGCTGC	GAGTTGATC	TCCACCGCTT	TGGGCTGTCT	TGATATTTCT	ATGTTCCGCA	
6850	6860	6870	6880	6890	6900	6910	6920	6930	6940
TTCCCGCTTCG	AAGTCCCTTC	GTGTTCCGAC	CTGTCCGCTT	ACCGATATCC	TGCTCCGCTT	TCTCCCTTCG	GGAAGCGTGC	CGCTTTTTCG	CGCTTTTTCG
AAGGGGAGCC	TTCCAGGGAG	CACGGAGAG	GACAGGCTG	GGACGGCGAA	TGGCTATGTC	ACAGGGCGAA	AGAGGGAGCA	CGCTTCGACC	
6960	6970	6980	6990	7000	7010	7020	7030	7040	7050
ATGCTACACG	TGTAGGTATC	TCAGTTGGT	GTAGGTCGTT	CGCTCCNAGC	TGGGCTGTGT	GCACCAACCC	CCCCTTCAGC	GGCGAAGTGC	GGCGAAGTGC
GCBAAGAGT	TACGAGTGCG	ACATCCATAG	AGTCAAGCCA	CATCCAGCAA	CGGAGGTTTC	ACCCGACACA	CGTCTTGGG	GGCGAAGTGC	
6950	6960	6970	6980	6990	7000	7010	7020	7030	7040
GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC	GGTAACATATC
GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC	GGTGGGAC
6940	6950	6960	6970	6980	6990	7000	7010	7020	7030
GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGGCTTACG	ACGGCTACAC	TAGAAAGGACA	GTATTTTGGTA	GTATTTTGGTA
CCTAATCTGC	TCGCTCCATA	CATCCGCCAC	GATCTCTCAA	GAACCTCACC	ACCGGTAATG	TGCCGATGTG	ATCTTCTCTG	CATAAACCAT	CATAAACCAT
7030	7040	7050	7060	7070	7080	7090	7100	7110	7120
CTGTAAGCCA	GTATACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG	GTAACCTTCG
CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT	CTGCGCTCTT
AGACCGGAGA	CGACTTCGGT	CAATGGAAGC	CTTTTTCTCA	ACCATCGAGA	ACTAGGCCGT	TTGTTTGGTG	GGGACCATCG	CCACCAAAAA	CCACCAAAAA
7120	7130	7140	7150	7160	7170	7180	7190	7200	7210
TTGTTTTCGA	GCACGAGATT	ACGCGAGAA	AAAAAGATC	TCAAGAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGTGCGA	CAGTGTGCGA
AACAAACGTT	CGTGTCTTAA	TGCGGTCTTT	TTTTTCTCTA	AGTTCTCTTA	GGAACTAGA	AAAGATGCC	CAGACTGCGA	GTCACTTCGC	GTCACTTCGC

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7210 AAACCTCAGC TTAAGGGATT 7220 TTGGTCATGA GATTATCAA 7230 AACCAGTACT CTAATAGTTT 7240 AAGGATCTTC ACCTAGATCC 7250 TTTAAATTA AAAATGAAGT 7260 TTTTAACTTA AAAATTAAT 7270 TTTTAACTTA AAAATTAAT 7280 TTTTAACTTA AAAATTAAT 7290 TTTTAACTTA AAAATTAAT
 7300 TTTTAACTTA AAAATTAAT 7310 TTTTAACTTA AAAATTAAT 7320 TTTTAACTTA AAAATTAAT 7330 TTTTAACTTA AAAATTAAT 7340 TTTTAACTTA AAAATTAAT 7350 TTTTAACTTA AAAATTAAT 7360 TTTTAACTTA AAAATTAAT 7370 TTTTAACTTA AAAATTAAT 7380 TTTTAACTTA AAAATTAAT 7390 TTTTAACTTA AAAATTAAT
 7400 TTTTAACTTA AAAATTAAT 7410 TTTTAACTTA AAAATTAAT 7420 TTTTAACTTA AAAATTAAT 7430 TTTTAACTTA AAAATTAAT 7440 TTTTAACTTA AAAATTAAT 7450 TTTTAACTTA AAAATTAAT 7460 TTTTAACTTA AAAATTAAT 7470 TTTTAACTTA AAAATTAAT 7480 TTTTAACTTA AAAATTAAT 7490 TTTTAACTTA AAAATTAAT
 7500 TTTTAACTTA AAAATTAAT 7510 TTTTAACTTA AAAATTAAT 7520 TTTTAACTTA AAAATTAAT 7530 TTTTAACTTA AAAATTAAT 7540 TTTTAACTTA AAAATTAAT 7550 TTTTAACTTA AAAATTAAT 7560 TTTTAACTTA AAAATTAAT 7570 TTTTAACTTA AAAATTAAT 7580 TTTTAACTTA AAAATTAAT 7590 TTTTAACTTA AAAATTAAT
 7600 TTTTAACTTA AAAATTAAT 7610 TTTTAACTTA AAAATTAAT 7620 TTTTAACTTA AAAATTAAT 7630 TTTTAACTTA AAAATTAAT 7640 TTTTAACTTA AAAATTAAT 7650 TTTTAACTTA AAAATTAAT 7660 TTTTAACTTA AAAATTAAT 7670 TTTTAACTTA AAAATTAAT 7680 TTTTAACTTA AAAATTAAT 7690 TTTTAACTTA AAAATTAAT
 7700 TTTTAACTTA AAAATTAAT 7710 TTTTAACTTA AAAATTAAT 7720 TTTTAACTTA AAAATTAAT 7730 TTTTAACTTA AAAATTAAT 7740 TTTTAACTTA AAAATTAAT 7750 TTTTAACTTA AAAATTAAT 7760 TTTTAACTTA AAAATTAAT 7770 TTTTAACTTA AAAATTAAT 7780 TTTTAACTTA AAAATTAAT 7790 TTTTAACTTA AAAATTAAT
 7800 TTTTAACTTA AAAATTAAT 7810 TTTTAACTTA AAAATTAAT 7820 TTTTAACTTA AAAATTAAT 7830 TTTTAACTTA AAAATTAAT 7840 TTTTAACTTA AAAATTAAT 7850 TTTTAACTTA AAAATTAAT 7860 TTTTAACTTA AAAATTAAT 7870 TTTTAACTTA AAAATTAAT 7880 TTTTAACTTA AAAATTAAT 7890 TTTTAACTTA AAAATTAAT
 7900 TTTTAACTTA AAAATTAAT 7910 TTTTAACTTA AAAATTAAT 7920 TTTTAACTTA AAAATTAAT 7930 TTTTAACTTA AAAATTAAT 7940 TTTTAACTTA AAAATTAAT 7950 TTTTAACTTA AAAATTAAT 7960 TTTTAACTTA AAAATTAAT 7970 TTTTAACTTA AAAATTAAT 7980 TTTTAACTTA AAAATTAAT 7990 TTTTAACTTA AAAATTAAT
 8000 TTTTAACTTA AAAATTAAT 8010 TTTTAACTTA AAAATTAAT 8020 TTTTAACTTA AAAATTAAT 8030 TTTTAACTTA AAAATTAAT 8040 TTTTAACTTA AAAATTAAT 8050 TTTTAACTTA AAAATTAAT 8060 TTTTAACTTA AAAATTAAT 8070 TTTTAACTTA AAAATTAAT 8080 TTTTAACTTA AAAATTAAT 8090 TTTTAACTTA AAAATTAAT 8100 TTTTAACTTA AAAATTAAT

Figure 14
(continued)

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8110      8120      8130      8140      8150      8160      8170      8180      8190
CCAGCGTTTC TGGGTGAGCA AAACACAGGAA GGCAAAATGC CGCAAAAGG GGAATPAGGG CGACACGGAA ATGTTGAATA CTCATACTCT
GGTCGCAAG ACCCACTCGT TTTTGTCCCT CCCTTTTACG GCGTTTTTTC CCTTATTCCC GCTGTGCCTT TACAACCTTAT GAGTATGAGA

8200      8210      8220      8230      8240      8250      8260      8270      8280
TCCTTTTTC AATATTATGA AGCATTTATC AGGTTTATG TCCTCATGAGC GGATACATAT TTGAATGTAT TTAGAAAAAT AAACAAATAG
AGGAAAAAGT TATNATAACT TCGTAAATAG TCCCAATAAC AGAGTACTCG CCTATGTATA AACTTATATA AATCTTTTAA TTGTTTATC

8290      8300      8310      8320      8330
GGGTTCGGG CACATTCCC CGAAAGTGC CACTGACGT C
CCCAAGGCGC GTGTAAAGG GCTTTTCAG GTGGACTGCA G
```

Figure 14
(continued)

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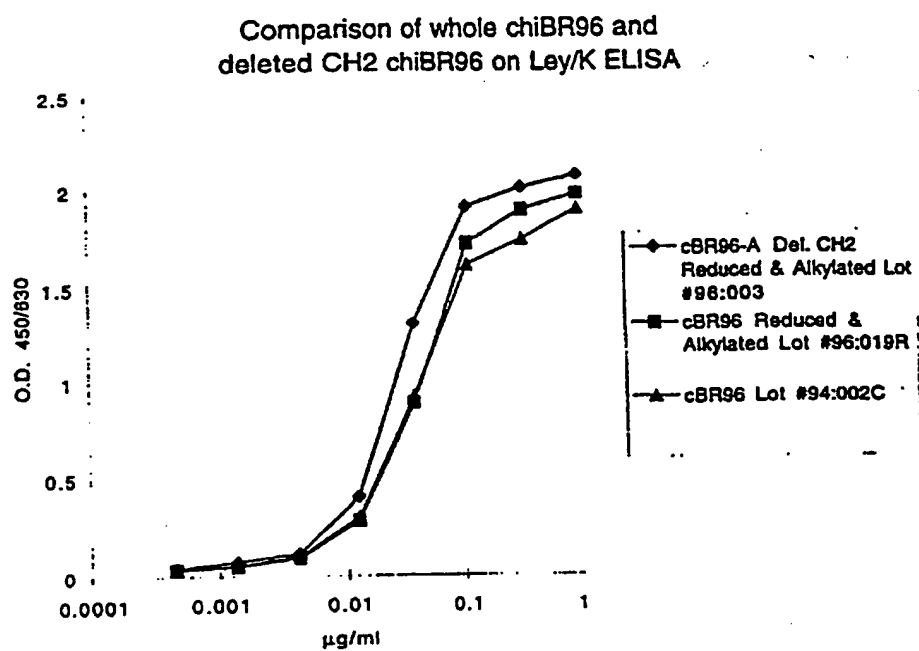


Figure 15

hBR96-2B: L235 to A235 and G237 to A237

hBR96-2C: E318 to S318, K320 to S320, and K322 to S322

hBR96-2D: P331 to A331

hBR96-2E: L235 to A235, G237 to A237, E318 to S318, K320 to S320, and K322 to S322

hBR96-2F: L235 to A235, G237 to A237, and P331 to A331

hBR96-2G: E318 to S318, K320 to S320, K322 to S322, and P331 to A331

hBR96-2H: L235 to A235, G237 to A237, E318 to S318, K320 to S320, K322 to S322, and P331 to A331

Figure 16

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FIGURE 17

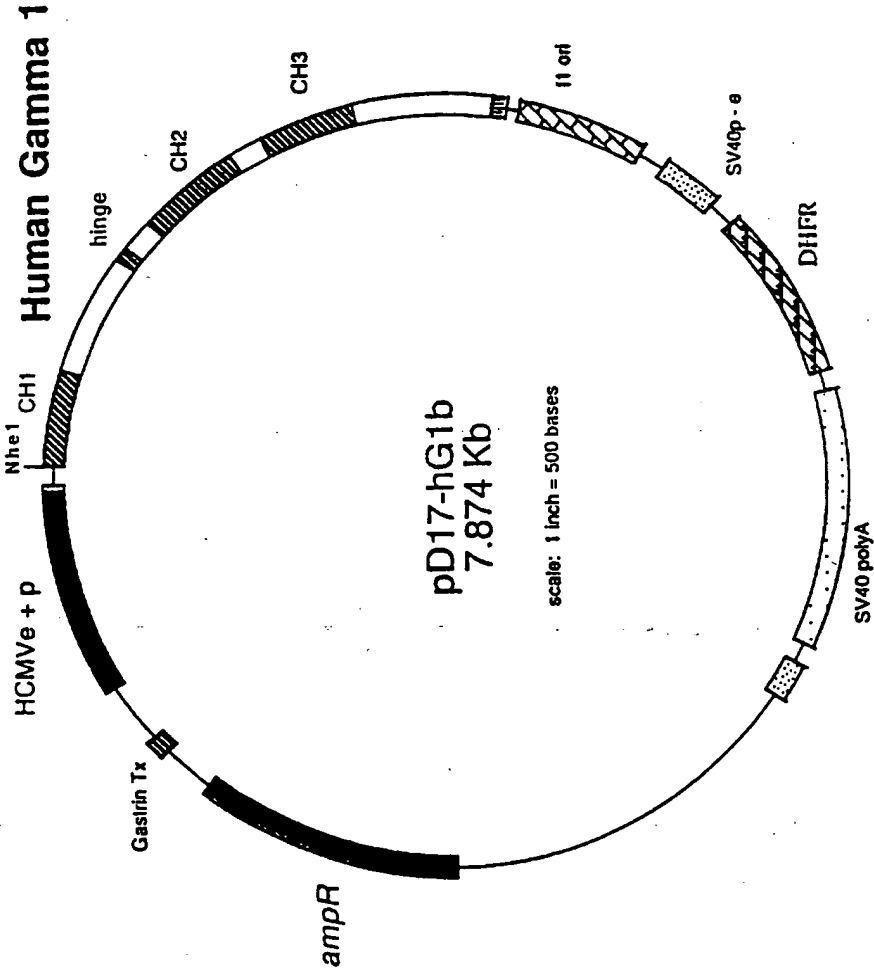


FIGURE 18A

1 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC
51 GGTCAATCGA TTGGAATCTT TGCGGCCGCT TGCTAGCCAC CATGGAGTTG
101 TGGTTAAGCT TGGTCTTCCT TGTCCTTGTT TTAAAAGGTG TCCAGTGTGA
151 AGTGCAACTG GTGGAGTCTG GGGGAGGCTT AGTGCAGCCT GGAGGGTCCC
201 TGCGACTTTC CTGTGCTGCA TCTGGATTCC CGTTCAGTGA CTATTACATG
251 TATTGGGTTC GCCAGGCTCC AGGCAAGGGA CTGGAGTGGG TCTCATACAT
301 TAGTCAAGAT GGTGATATAA CCGACTATGC AGACTCCGTA AAGGGTCGAT
351 TCACCATCTC CAGAGACAAT GCAAAGAACA GCCTGTACCT GCAAATGAAC
401 AGCCTGAGGG ACGAGGACAC AGCCGTGTAT TACTGTGCAA GAGGCCTGGC
451 GGACGGGGCC TGGTTTGCTT ACTGGGGCCA AGGGACTCTG GTCACGGTCT
501 CTTCCGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
551 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA
601 CTTCCCCGAA CCGGTGACGG TGTCGTGGAA CTCAGGCGCC CTGACCAGCG
651 GCGTGACAC CTTCCCGGCT GTCCTACAGT CCTCAGGACT CTACTCCCTC
701 AGCAGCGTGG TCACCGTGCC CTCCAGCAGC TTGGGCACCC AGACCTACAT
751 CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGTTG
801 GTGAGAGGCC AGCACAGGGA GGGAGGGTGT CTGCTGGAAG CCAGGCTCAG
851 CGCTCCTGCC TGGACGCATC CCGGCTATGC AGCCCCAGTC CAGGGCAGCA
901 AGGCAGGCCC CGTCTGCCTC TTCACCCGGA GGCCTCTGCC CGCCCCACTC
951 ATGCTCAGGG AGAGGGTCTT CTGGCTTTTT CCCCAGGCTC TGGGCAGGCA
1001 CAGGCTAGGT GCCCCTAACC CAGGCCCTGC ACACAAAGGG GCAGGTGCTG
1051 GGCTCAGACC TGCCAAGAGC CATATCCGGG AGGACCCTGC CCCTGACCTA
1101 AGCCCACCCC AAAGGCCAAA CTCTCCACTC CCTCAGCTCG GACACCTTCT
1151 CTCTCCAG ATTCCAGTAA CTCCCAATCT TCTCTCTGCA GAGCCCCAAT
1201 CTTGTGACAA AACTCACACA TGCCCACCGT GCCCAGGTAA GCCAGCCCAG
1251 GCCTCGCCCT CCAGCTCAAG GCGGGACAGG TGCCCTAGAG TAGCCTGCAT
1301 CCAGGGACAG GCCCCAGCCG GGTGCTGACA CGTCCACCTC CATCTCTTCC

1351 TCAGCACCTG AACTC²³⁵CTGGG ²³⁷GGGCCGTCA GTCTTCCTCT TCCCCCAAA
 1401 ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
 1451 TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
 1501 GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
 1551 CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
 1601 GGCTGAATGG CAAG³¹⁸CAGTAC ³²⁰AAGTGC³²²ACG TCTCCAACAA AGCCCTCCCA
 1651 ³³¹GCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGTGGGA CCCGTGGGGT
 1701 GCGAGGGCCA CATGGACAGA GGCCGGCTCG GCCCACCTC TGCCCTGAGA
 1751 GTGACCGCTG TACCAACCTC TGTCCCTACA GGGCAGCCCC GAGAACCACA
 1801 GGTGTACACC CTGCCCCCAT CCCGGGATGA GGTGACCAAG AACCAGGTCA
 1851 GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
 1901 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT
 1951 GCTGGACTCC GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA
 2001 AGAGCAGGTG GCAGCAGGGG AACGTCTTCT CATGCTCCGT GATGCATGAG
 2051 GCTCTGCACA ACCACTACAC GCAGAAGAGC CTCTCCCTGT CTCCGGGTAA
 2101 ATGAGTGCGA CGGCCGGCAA GCCCCGCTC CCCGGGCTCT CGCGGTCGCA
 2151 CGAGGATGCT TGGCACGTAC CCCCTGTACA TACTTCCCGG GCGCCCAGCA
 2201 TGGAAATAAA GCACCCAGCG CTGCCCTGGG CCCCTGCGAG ACTGTGATGG
 2251 TTCTTTCCAC GGGTCAGGCC GAGTCTGAGG CCTGAGTGGC ATGAGGGAGG
 2301 CAGAGCGGGT CCCACTGTCC CCACACTGGC CCAGGCTGTG CAGGTGTGCC
 2351 TGGGCCCCCT AGGGTGGGGC TCAGCCAGGG GCTGCCCTCG GCAGGGTGGG
 2401 GGATTTGCCA GCGTGGCCCT CCCTCCAGCA GCACCTGCCC TGGGCTGGGC
 2451 CACGGGAAGC CCTAGGAGCC CTGGGGACA GACACACAGC CCCTGCCTCT
 2501 GTAGGAGACT GTCCTGTTCT GTGAGCGCCC CTGTCCTCCC GACCTCCATG
 2551 CCCACTCGGG GGCATGCCTA GTCCATGTGC GTAGGGACAG GCCCTCCCTC
 2601 ACCCATCTAC CCCCACGGCA CTAACCCCTG GCTGCCCTGC CCAGCCTCGC
 2651 ACCCGCATGG GGACACAACC GACTCCGGGG ACATGCACTC TCGGGCCCTG
 2701 TGGAGGGACT GGTGCAGATG CCCACACACA CACTCAGCCC AGACCCGTTT
 2751 AACAAACCCC GCACTGAGGT TGGCCGGCCA CACGGCCACC ACACACACAC
 2801 GTGCACGCCT CACACACGGA GCCTCACCCG GGCGAACTGC ACAGCACCCA

FIGURE 18B

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2851 GACCAGAGCA AGGACCTCGC ACACGTGAAC ACTCCTCGGA CACAGGCCCC
2901 CACGAGCCCC ACGCGGCACC TCAAGGCCCA CGAGCCTCTC GGCAGCTTCT
2951 CCACATGCTG ACCTGCTCAG ACAAACCCAG CCCTCCTCTC ACAAGGGTGC
3001 CCCTGCAGCC GCCACACACA CACAGGGGAT CACACACCAC GTCACGTCCC
3051 TGGCCCTGGC CCACTTCCCA GTGCCGCCCT TCCCTGCAGG ACGGATCAGC
3101 CTCGACTGTG CTTTCTAGTT GCCAGCCATC TGTGTGTTGC CCCTCCCCCG
3151 TGCCTTCCTT GACCCTGGAA GGTGCCACTC CCACTGTCCT TTCCTAATAA
3201 AATGAGGAAA TTGCATCGCA TTGTCTGAGT AGGTGTCATT CTATTCTGGG
3251 GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTGGGAA GACAATAGCA
3301 GGCATGCTGG GGATGCGGTG GGCTCTATGG CTTCTGAGGC GGAAAGAACC
3351 AGCTGGGGCT CTAGGGGGTA TCCCCACGCG CCCTGTAGCG GCGCATTAAG
3401 CGCGGCGGGT GTGGTGGTTA CGCGCAGCGT GACCGCTACA CTTGCCAGCG
3451 CCCTAGCGCC CGCTCCTTTC GCTTTCTTCC CTTCCCTTCT CGCCACGTTC
3501 GCCGGGCCTC TCAAAAAGG GAAAAAAGC ATGCATCTCA ATTAGTCAGC
3551 AACCATAGTC CCGCCCCCTAA CTCCGCCCAT CCCGCCCCCTA ACTCCGCCCCA
3601 GTTCCGCCCA TTCTCCGCCC CATGGCTGAC TAATTTTTTTT TATTTATGCA
3651 GAGGCCGAGG CCGCCTCGGC CTCTGAGCTA TTCCAGAAGT AGTGAGGAGG
3701 CTTTTTTGGA GGCCTAGGCT TTTGCAAAA GCTTGGACAG CTCAGGGCTG
3751 CGATTTTCGCG CCAAACTTGA CGGCAATCCT AGCGTGAAGG CTGGTAGGAT
3801 TTTATCCCCG CTGCCATCAT GGTTGACCA TTGAACTGCA TCGTCGCCGT
3851 GTCCCAAAT ATGGGGATTG GCAAGAACGG AGACCTACCC TGGCCTCCGC
3901 TCAGGAACGA GTTCAAGTAC TTCCAAAGAA TGACCACAAC CTCTTCAGTG
3951 GAAGGTAAAC AGAATCTGGT GATTATGGGT AGGAAAACCT GGTTCCTCAT
4001 TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA
4051 GAGAACTCAA AGAACCACCA CGAGGAGCTC ATTTTCTTGC CAAAAGTTTG
4101 GATGATGCCT TAAGACTTAT TGAACAACCG GAATTGGCAA GTAAAGTAGA
4151 CATGGTTTGG ATAGTCGGAG GCAGTTCTGT TTACCAGGAA GCCATGAATC
4201 AACCAGGCCA CCTTAGACTC TTTGTGACAA GGATCATGCA GGAATTTGAA
4251 AGTGACACGT TTTTCCCAGA AATTGATTG GGGAAATATA AACTTCTCCC
4301 AGAATACCCA GGCGTCCTCT CTGAGGTCCA GGAGGAAAAA GGCATCAAGT

4351 ATAAGTTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTTCAAG
4401 TTCTCTGCTC CCCTCCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT
4451 TTGCTGGCTT TAGATCTCTT TGTGAAGGAA CCTTACTTCT GTGGTGTGAC
4501 ATAATTGGAC AAACCTACCTA CAGAGATTTA AAGCTCTAAG GTAAATATAA
4551 AATTTTTAAG TGTATAATGT GTTAAACTAC TGATTCTAAT TGTTTGTGTA
4601 TTTTAGATTC CAACCTATGG AACTGATGAA TGGGAGCAGT GGTGGAATGC
4651 CTTTAATGAG GAAAACCTGT TTTGCTCAGA AGAAATGCCA TCTAGTGATG
4701 ATGAGGCTAC TGCTGACTCT CAACATTCTA CTCCTCCAAA AAAGAAGAGA
4751 AAGGTAGAAG ACCCCAAAGGA CTTTCCTTCA GAATTGCTAA GTTTTTTGAG
4801 TCATGCTGTG TTTAGTAATA GAACTCTTGC TTGCTTTGCT ATTTACACCA
4851 CAAAGGAAAA AGCTGCACTG CTATACAAGA AAATTATGGA AAAATATTCT
4901 GTAACCTTTA TAAGTAGGCA TAACAGTTAT AATCATAACA TACTGTTTTT
4951 TCTTACTCCA CACAGGCATA GAGTGTCTGC TATTAATAAC TATGCTCAAA
5001 AATTGTGTAC CTTTAGCTTT TTAATTTGTA AAGGGGTTAA TAAGGAATAT
5051 TTGATGTATA GTGCCCTGAC TAGAGATCAT AATCAGCCAT ACCACATTTG
5101 TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG
5151 AAACATAAAA TGAATGCAAT TGTGTGTGTT AACTGTTTA TTGCAGCTTA
5201 TAATGGTTAC AAATAAAGCA ATAGCATCAC AAATTCACA AATAAAGCAT
5251 TTTTTTCACT GCATTCTAGT TGTGGTTTGT CCAAATCAT CAATGTATCT
5301 TATCATGTCT GGATCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT
5351 GGAGTTCTTC GCCCACCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA
5401 AATAAAGCAA TAGCATCACA AATTTACAA ATAAAGCATT TTTTCACTG
5451 CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG
5501 TATACCGTCG ACCTCTAGCT AGAGCTTGGC GTAATCATGG TCATAGCTGT
5551 TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACGAGCC
5601 GGAAGCATAA AGTGTAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC
5651 ATTAATTGCG TTGCGCTCAC TGCCCGCTTT CCAGTCGGGA AACCTGTCGT
5701 GCCAGCTGCA TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCCT
5751 ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC GCTCGGTCGT
5801 TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT

5351 CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG
5901 CAAAAGGCCA GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG
5951 GCTCCGCCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT
6001 GGCGAAACCC GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC
6051 TCCCTCGTGC GCTCTCCTGT TCCGACCCGT CCGCTTACCG GATACCTGTC
6101 CGCCTTTCTC CCTTCGGGAA GCGTGGCGCT TTCTCAATGC TCACGCTGTA
6151 GGTATCTCAG TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC
6201 GAACCCCCCG TTCAGCCCGA CCGCTGCGCC TTATCCGGTA ACTATCGTCT
6251 TGAGTCCAAC CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG
6301 GTAACAGGAT TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG
6351 AAGTGGTGGC CTAACCTACG CTACACTAGA AGGACAGTAT TTGGTATCTG
6401 CGCTCTGCTG AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT
6451 CCGGCAAACA AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG
6501 CAGATTACGC GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC
6551 TACGGGGTCT GACGCTCAGT GGAACGAAAA CTCACGTTAA GGGATTTTGG
6601 TCATGAGATT ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATTAAAAA
6651 TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT GGTCTGACAG
6701 TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC
6751 GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG
6801 GAGGGCTTAC CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG
6851 CTCACCGGCT CCAGATTTAT CAGCAATAAA CCAGCCAGCC GGAAGGGCCG
6901 AGCGCAGAAG TGGTCCTGCA ACTTTATCCG CCTCCATCCA GTCTATTAAT
6951 TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA GTTTGCGCAA
7001 CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGTA
7051 TGGCTTCATT CAGCTCCGGT TCCCAACGAT CAAGGCGAGT TACATGATCC
7101 CCCATGTTGT GCAAAAAGC GGTTAGCTCC TTCGGTCCTC CGATCGTTGT
7151 CAGAAGTAAG TTGGCCGCAG TGTTATCACT CATGGTTATG GCAGCACTGC
7201 ATAATTCTCT TACTGTCATG CCATCCGTAA GATGCTTTTC TGTGACTGGT
7251 GAGTACTCAA CCAAGTCATT CTGAGAATAG TGTATGCGGC GACCGAGTTG
7301 CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT

FIGURE 18E

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7351 TAAAAGTGCT CATCATTGGA AAACGTTCTT CGGGGCGAAA ACTCTCAAGG
7401 ATCTTACCGC TGTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA
7451 CTGATCTTCA GCATCTTTTA CTTTCACCAG CGTTTCTGGG TGAGCAAAAA
7501 CAGGAAGGCA AAATGCCGCA AAAAAGGGAA TAAGGGCGAC ACGGAAATGT
7551 TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA TTTATCAGGG
7601 TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC
7651 AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCGAC
7701 GGATCGGGAG ATCTGCTAGG TGACCTGAGG CGCGCCGGCT TCGAATAGCC
7751 AGAGTAACCT TTTTTTTTAA TTTTATTTTA TTTTATTTT GAGATGGAGT
7801 TTGGCGCCGA TCTCCCGATC CCCTATGGTC GACTCTCAGT ACAATCTGCT
7851 CTGATGCCGC ATAGTTAAGC CAGTATCTGC TCCCTGCTTG TGTGTTGGAG
7901 GTCGCTGAGT AGTGCGCGAG CAAATTTTAA GCTACAACAA GGCAAGGCTT
7951 GACCGACAAT TGCATGAAGA ATCTGCTTAG GGTAGGCGT TTTGCGCTGC
8001 TTCGCGATGT ACGGGCCAGA TATACGCGTT GACATTGATT ATTGACTAGT
8051 TATTAATAGT AATCAATTAC GGGGTCATTA GTTCATAGCC CATATATGGA
8101 GTTCCGCGTT ACATAACTTA CGGTAAATGG CCCGCCTGGC TGACCGCCCA
8151 ACGACCCCCG CCCATTGACG TCAATAATGA CGTATGTTCC CATAGTAACG
8201 CCAATAGGGA CTTTCCATTG ACGTCAATGG GTGGACTATT TACGGTAAAC
8251 TGCCCCACTTG GCAGTACATC AAGTGTATCA TATGCCAAGT ACGCCCCCTA
8301 TTGACGTCAA TGACGGTAAA TGGCCCGCCT GGCATTATGC CCAGTACATG
8351 ACCTTATGGG ACTTTCCTAC TTGGCAGTAC ATCTACGTAT TAGTCATCGC
8401 TATTACCATG GTGATGCGGT TTTGGCAGTA CATCAATGGG CGTGATAGC
8451 GGTTTGACTC ACGGGGATTT CCAAGTCTCC ACCCCATTGA CGTCAATGGG
8501 AGTTTGTTTT GGCACCAAAA TCAACGGGAC TTTCCAAAAT GTCGTAACAA
8551 CTCCGCCCCA TTGACGCAAA TGGGCGGTAG GCGTGTACGG TGGGAGGTCT
8601 ATATAAGCAG AGCTCTCTGG CTAAGTAGAG AACCCACTGC TTACTGGCTT
8651 ATCGAAATTA ATACGACTCA CTATAGGGAG ACCCAAGCTT

FIGURE 18F

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FIGURE 19 A

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10 GGTACCAATT TAAATTGATA TCTCCTTAGG TCTCGAGTCT CTAGATAACC GGTCAATCGA 60
CCATGGTTAA ATTTAACTAT AGAGGAATCC AGAGCTCAGA GATCTATTGG CCAGTTAGCT
70 TTGGAATTC TCGGGCCGCT TGCTAGCACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC
AACCTTAAGA ACGCCGGCGA ACGATCGTGG TTCCCGGGTA GCCAGAAGGG GGACCGTGGG
130 TCCCTCCAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT GCCTGGTCAA GGACTACTTC
AGGAGCTTCT CGTGGAGACC CCGGTGTCG CCGGACCCGA CGGACCAAGT CCTGATGAAG
190 CCGGAACCGG TGACGGTGTG GTGGAATCA GCGGCCCTGA CCAGCGGCGT GCACACCTTC
GGGCTTGCC ACTGCCACAG CACTTGAGT CCGCGGACT GGTGCGCCGA CGTGTGAAG
250 CCGGCTGTC TACAGTCTC AGGACTCTAC TCCCTCAGCA GCGTGGTCA CGTGCCCTCC
GGCCGACAG ATGTCAGGAG TCCTGAGATG AGGGAGTGT CCGACCAAGT GCACGGGAGG
310 AGCAGCTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCAG CAACACCAAG
TCGTGCAACC CGTGGGTCG GATGTAGACG TTGCACTTAG TGTTCGGTC GTTGTGTTTC
370 G'GGACAAGA AAGTGTGTA GAGGCCAGCA CAGGAGGGA GGGTGTCTGC TGAAGCCAG
CACCTGTCT 'TTCAACCACT CTCCGGTCTG GTCCCTCCCT CCCACAGAG ACCTTCGGTC
430 GCTCAGCGT CCTGCCTGA CCGATCCCG CTA'GCGAGC CCAGTCCAG GCAGCAAGC
CGAGTCGCA GGACGGACCT GCGTAGGGCC GATACGTCGG GGTACAGTCC CGTCGTTCG
490 AGGCCCGTC TGCCTCTCA CCGGAGGCC TCTGCCGCC CCACCTATGC TCAGGGAGG
TCCGGGSCAG ACGGAGAAGT GGGCTCCCG AGACGGCGG GGTGAGTAC AGTCCCTCTC
550 GGTCTTCTG CTTTTTCCC AGGCTCTGG CAGGCACAG CTAGGTGCCC CTAACCCAGG
CCACACAC ACC GAAAAGGGG TCCGAGACCC GTCCG'CTTC GATCCACGG GATTGGGTCC 600

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FIGURE 19B

pD17-hG1b

610 620 630 640 650 660
 CCCTGCACAC AAAGGGCAG GTGCTGGGT CAGACTGCC AAGAGCCATA TCCGGGAGGA
 GGGACGTGTG TTTCCCGCTC CACGACCCGA GTCTGGACGG TTCTCGGTAT AGGCCCTCCT
 670 680 690 700 710 720
 CCCTGCCCTT GACCTAAGCC CACCCCAAG GCCAACTCT CCACTCCCTC AGCTCGGACA
 GGGACGGGA CTGGATTGG GTGGGTTTC CGTTTGAGA GGTGAGGGAG TCGAGCCTGT
 730 740 750 760 770 780
 CCTTCTCTCC TCCAGATTC CAGTAATCC CAATCTTTC TCTGCAGAGC CCAANTCTTG
 GGAAGAGAGG AGGCTCTAAG GTCATTTGAGG GTTAGAAGAG AGACGTCTCG GGTTTAGAAC
 790 800 810 820 830 840
 TGACAAACT CACACATGCC CACCGTGCC AGGTAAGCCA GCCCAGGCCT CGCCCTCCAG
 ACTGTATTGA GTGTCTACGG GTGGCACGG TCCATTCCGT CCGGTCCGGA GCGGAGGTC
 850 860 870 880 890 900
 CTCAGGCGG GACAGTGCC CTAGAGTAGC CTGCATCCAG GGACAGGCC CAGCCGGGTG
 GAGTCCCGC CTGTCCACGG GATCTCATCG GACGTAGTC CCTGTCCGG GTCCGCCAC
 910 920 930 940 950 960
 CTCACACGTC CACCTCCATC TCTTCTCTCAG CACCTGAAC CTGTGGGGA CCGTCAGTCT
 GACTGTCCAG GTGGAGGTAG AGAAGGATC GTGGACTTGA GACTCCCTT GGCAGTCAGA
 970 980 990 1000 1010 1020
 TCTCTTTCCC CCCAAACCC AAGACACCC TCATGATCTC CCGACCCCT GAGTCAAT
 AGGAGAGGG GGGTTTGGG TTCTGTGGG AGTAC"AGAG GCCCTGGGA CTCAGTSTA
 1030 1040 1050 1060 1070 1080
 GCGTGGTGGT GGACGTGAGC CACGAAGACC CTGAGGTCAA GTTCAACTGG TACGTGGAGC
 CGCACCAACA CCTGCACCTG GTGCTTCTGG GACTCCAGTT CAAGTTGACC ATGCACCTGC
 1090 1100 1110 1120 1130 1140
 CCGTGGAGGT GCATAATGCC AAGACAAAG CGCGGGAGA GCAGTACAC AGCACGTACC
 CGCACCTTCA CGTATTACGG TTCTGTCTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG
 1150 1160 1170 1180 1190 1200
 GTGTGGTTCAG CGTCTCACC GTCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAGT
 CACACCTCAGT GCAGGAGTGG CAGGACGTGG TCCTGACCCA CT"ACCGTTC CTCATGTTCA

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FIGURE 19C

pD17-hG1b

322 1210 1220 1230 1240 1250 1260
 GCAAGGCTTC CAACAAAGCC CTCCAGGCC CCATCGAGAA AACCATCTCC AAAGCCAAAG
 GGTCCAGAG GTTGTTCGG GAGGTCGGG GGTAGCTCTT TTGGTAGAGG TTTCGGTTC
 1270 1280 1290 1300 1310 1320
 GTGGACCCG TGGGTGCGA GGGCCACATG GACAGAGGCC GGCTCGGCC ACCCTCTGCC
 CACCCCTGGC ACCCCACGCT CCCGGTGTAC CTGTCTCCGG CCGAGCCGG TGGGAGACGG
 1330 1340 1350 1360 1370 1380
 CTGAGAGTGA CCGCTGTACC AACCTCTGTC CCTACAGGGC AGCCCCGAGA ACCACAGGTG
 GACTCTCACT GCGACATGG TTGGAGACAG GGATGTCCCG TCGGGGCTCT TGGTGTCCAC
 1390 1400 1410 1420 1430 1440
 TACACCCCTGC CCCCATCCG GGATGAGCTG ACCAAGAAC AGGTCAGCCT GACCTGCCCTG
 ATGTGGGACG GGGTAGGGC CCTACTCGAC TGGTCTTGG TCCAGTCGGA CTGGACGGAC
 1450 1460 1470 1480 1490 1500
 GTCAAGGGCT TCTATCCAG CGACATCGCC GTGGAGTGG AGAGCAATGG GCAGCCGGAG
 CAGTTTCCGA AGATAGGGTC GCTGTAGCG CACCTCACCC TCTCGTTACC CGTCGGCCTC
 1510 1520 1530 1540 1550 1560
 AACRACTACA AGACCAGCC TCCCGTCTG GACTCCGACG GCTCCTTCTT CCTCTACAGC
 TTGTGTGATG TCTGGTCCG AGGGCAGGAC CTGAGGCTGC CGAGGAAGAA GGAGATGTG
 1570 1580 1590 1600 1610 1620
 AAGCTCACCG TGGACAAGAG CAGGTGGCAG CAGGGGAACG TCTTCTCATG CTCCGTGATG
 TTCGAGTGGC ACCTGTCTC GTCCACCGTC GTCCCTTGC AGAAGAGTAC GAGGCACTAC
 1630 1640 1650 1660 1670 1680
 CATGAGGCTC TGCACAACCA CTACACGCAG AAGAGCTTCT CCCTGTCTCC GGTAAATGA
 GTACTCCGAG ACGTGTGGT GATGTCCGTC TTCTCGGAGA GGGACAGAGG CCCATTACT
 1690 1700 1710 1720 1730 1740
 GTGCGACGGC CGCAAGCCC CCGTCCCGG GGCTCTCGG GTCCACAGAG GATGCTTGGC
 CACGCTGCCG GCGTTCGGG GCGAGGGGC CCGAGAGGCG CAGCGTCTC CTACGAACCG
 1750 1760 1770 1780 1790 1800
 ACGTACCCCC TGTACATACT TCCCGGGCGC CCAGCATGGA AATAAGCAC CCAGCGCTGC
 TGCATGGGGG ACATGTATGA AGGGCCCGG GGTCTGTACCT TTATTTCTG GGTGCGACG

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FIGURE 19D

pD17-hG1b

1810	1820	1830	1840	1850	1860
CCCTGGCCCC	TGCAGACATG	TGATGGTTCT	TTCCACGGGT	CAGGCCGAGT	CTGAGGCCCTG
GGACCCGGGG	ACGCTCTGAC	ACTACCAAGA	AAGGTGCCCA	GTCCGGCTCA	GACTCCGGAC
1870	1880	1890	1900	1910	1920
AGTGGCAIGA	GGGAGGCAGA	GCGGGTCCCA	CTGTCCCCAC	ACTGGCCACG	GCTGTGCAGG
TCACCGTACT	CCCTCCGTCT	CGCCACGGGT	GACAGGGGTG	TGACCCGGTC	CGACACGTCC
1930	1940	1950	1960	1970	1980
TGTCCCTGGG	CCCCCTAGGG	TGGGGCTCAG	CCAGGGGCTG	CCCTCGGCAG	GGTGGGGGAT
ACACGGACCC	GGGGGATCCC	ACCCCGAGTC	GGTCCCCGAC	GGGAGCCGTC	CCACCCCTTA
1990	2000	2010	2020	2030	2040
TTGCCAGCGT	GGCCCTCCCT	CCAGCAGCAC	CTGCCCTGGG	CTGGGCCACG	GGAAAGCCCTA
AACGGTCGCA	CCGGGAGGGA	GGTCGTCTGT	GACGGGACCC	GACCCGGTGC	CCTTCGGGAT
2050	2060	2070	2080	2090	2100
GGAGCCCTGT	GGGACAGACA	CACAGCCCTT	GCCTCTCTAG	GAGACTGTCC	TGTTCTGTGA
CCTCGGGGAC	CCCTGTCTGT	G'GTCTCGGGA	CGGAGACATC	CTCTGACAGG	ACAAGACACT
2110	2120	2130	2140	2150	2160
GCGCCCTGT	CTTCCCGACC	TCCATGCCCA	CTCGGGGGCA	TGCTGGGGAT	GCGGTGGGCT
CGCGGGGACA	GGAGGCTGG	AGGTACGGGT	GAGCCCCCGT	ACGACCCCTA	CGCCACCCGA
2170	2180	2190	2200	2210	2220
C'TA'ITGG'ITC	TGAGGCGGAA	AGAACCAAGT	GGGGCTCTAG	GGGGTATCCC	CACGGCCCTT
GATACCGAAG	ACTCCGCCCT	TCTTGTGCGA	CCCCGAGATC	CCCCATAGGG	GTGCGGGGGA
2230	2240	2250	2260	2270	2280
GTAGCGGCGC	ATTAAAGCGG	GCGGGTGTGG	TGGTTACGCG	CAGCGTGACC	GCTACACTTG
CATCGCCCGC	TAAITTCGCG	CGCCACACAC	ACCAATGCGC	GTGCGACTGG	CGATGTGAAC
2290	2300	2310	2320	2330	2340
CCAGCGCCCT	AGCGCCGCT	CCTTTCGCTT	TCTTCCCTTC	CTTTCTCGCC	ACGTTGCGCG
GGTCGCGGGA	TCGCGGGCGA	GGAAAGCGAA	AGAAGGGGAG	GAAAGAGCGG	TGCAAGCGGC
2350	2360	2370	2380	2390	2400
GCTTTCCCGG	TCAAGCTCTA	AATCGGGGCA	TCCCTTTAGG	GTTCGGATTT	ACTGCTTTAC
CGAAAGGGC	AGTTCGAGAT	TTAGCCCCCG	AGGGAATCC	CAAGGCTAAA	TCACGAAATG

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FIGURE 19E

pD17-hG1b

2410 2420 2430 2440 2450 2460
 GGCACCTCGA CCCCAAAAA CTGATAGG GTGATGGTTC ACGTAGTGG CCATCGCCCT
 CCGTGGAGCT GGGGTTTTTTT GAACATAATCC CACTACCAAG TGCATCACCC GGTAGCGGGA

 2470 2480 2490 2500 2510 2520
 GATGACGGT TTTTCGCCCT TTGACGTGG AGTCCACGTT CTTTAATAGT GGACTCTTGT
 CTATCTGCCA AAAAGCGGA AACTGCNACC TCAGGTGCA GAAATTATCA CTTGAGAACA

 2530 2540 2550 2560 2570 2580
 TCCAAACATGG AACAACTC AACCTATCT CCGTCTATTC TTTTGATTTA TAAGGGATTT
 AGGTTTGACC TTGTTGTGAG TTGGGATAGA GCCAGATAAG AAAACTAAAT ATTCCCTAAA

 2590 2600 2610 2620 2630 2640
 TGGGATTTTC GGCCTATTGG TTAAAAATG AGCTGATTTA ACAAAAATTT AACGGGAATTT
 ACCCTTAAAG CCGGATTAACC AATTTTTTAC TCGACTAAAT TGTTTTTTAA TTGCGCTTAA

 2650 2660 2670 2680 2690 2700
 AATCTGTGG AATGTGTTC AGTTAGGTG TGGAAAGTCC CCAGGCTCCC CAGGCAGGCA
 TTAAGACACC TTACACACAG TCAATCCCAC ACCTTTCAGG GTCCGAGGG GTCCGTCCGT

 2710 2720 2730 2740 2750 2760
 GAAGTATGCA AAGCATGCAT CTCATTTAGT CAGCAACCAT AGTCCCGCCC CTAACCTCCG
 CTTCAATACGT TTCTGTACGTA GAGTTAATCA GTCTGTGGTA TCAGGCGGG GATTGAGCG

 2770 2780 2790 2800 2810 2820
 CCATCCCGCC CCTAATCCG CCCAGTTCCG CCCATTTCTCC GCCCATGGC TGACTAATTT
 GGTAGGGCGG GGATTGAGG GGGTCAAGG GGGTAAGAGG CCGGTACCG ACTGATTTAA

 2830 2840 2850 2860 2870 2880
 TTTTATTATTA TGCAGAGGC GAGGCGCCT CGGCTCTGA GCTATTCCAG AAGTAGTGAG
 AAAAATAAAT ACGTCTCCG CTCCGGCGGA GCCGAGACT CGATAAGGTC TTCAATCACTC

 2890 2900 2910 2920 2930 2940
 GAGGCTTTT TGGAGGCCTA GGTTTTGA AAAAGCTGG ACAGCTCAGG GCTGCGATTT
 CTCCGAAAA ACCTCCGGAT CCGAAAAAGT TTTTCCGAACC TGTCGAGTCC CGACGCTAAA

 2950 2960 2970 2980 2990 3000
 CGCGCCAAAC TTGACGGCAA TCCTAGCGTG AAGGCTGGTA GGATTTTATC CCCGCTGCCA
 GCGCCCTTTT AACTGCCGTT AGGATCGCAC TTCCGACCAT CCTAAAAAG GCGCGACGCT

FIGURE 19F

pD17-hG1b

3010 TCATGGTTCG ACCATTGAAC TGCATCGTCG CCGTCGCCA AAATATGGGG ATTGGCAAGA 3060
AGTACCAAGC TGGTAACCTG ACGTAGCAGC GGCACAGGGT TTTATACCCC TAACCGTTCT
3070 ACGGAGACCT ACCCTGGCCT CCGCTCAGGA ACGAGTTCAA GTACTTCCAA AGAATGACCA 3120
TGCC'TCTGGA TGGGACCGGA GCGAGTCCT TGCTCAAGTT CATGAAGGTT TCTTACTGGT
3130 CAACCTCTTC AGTGGAGGT AAACAGATC TGGTGTATAT GGTAGGAAA ACCTGGTTCT 3180
GTTGGAGAAG TCACCTTCCA TTTGTCTTAG ACCACTAATA CCCATCCCTT TGGACCAAGA
3190 CCATTCCTGA GAAGAATCGA CCTTAAAGG ACAGAAATTA TATAGTTCTC ACTAGAGAAC 3240
GGTAAGGACT CTCTTAGCT GGAATTTCC TGCTTAAAT ATATCAAGAG TCATCTCTTG
3250 TCAAAGAACC ACCAGGAGGA GCTCATTTTC TTGCCAAAAG TTTGGATGAT GCCTPAAGAC 3300
AGTTCTCTGG TGGTGTCTCT CGAGTAAAG AACGGTTTC AACCTACTA CGGAATTTCTG
3310 TTATTGAACA ACCGGAATTG GCAAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT 3360
AATAACTTGT TGGCCTTAAC CGTTCATTTT ATCTGTACCA AACCTATCAG CCTCCGTCAA
3370 CTGTTTACCA GGAAGCCATG AATCAACCAG GCCACCTTAG ACTCTTTGTG ACAAGGATCA 3420
GACAAATGGT CTTTCGGTAC TTAGTTGGTC CCGTGGAAATC TGAGAAACAC TGTTCCCTAGT
3430 TGCAGGAAT TGAAGTGAC ACGTTTTC CAGAAATGA TTTGGGAAA TATAAACTTC 3480
ACGTCTCTAA ACTTTCACCTG TGCAAAAGG GTCTTAACT AAACCCCTTT ATATTTGAAG
3490 TCCCAGAATA CCCAGGCGTC CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT 3540
AGGGTCATAT GGGTCCCGCAG GAGAGACTCC AGGTCCCTCT TTTTCCCTAG TTCATATTTCA
3550 TTGAAGTCTA CGAGAAGAAA GACTAACAGG AAGATGCTTT CAAGTTCTCT GCTCCCTCC 3600
NACTCAGAT GCTCTCTCTT CTGATTGCTC TTCTACGAAA GTTCAAGAGA CGAGGGGAGG

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FIGURE 19C

pD17-hG1b

3610 3620 3630 3640 3650 3660
TAAAGCTATG CATTTTATATA AGACCATGGG ACTTTTGCTG GCTTTAGATC TCTTTGTGAA
ATTTCGATAC GTAAAAATAT TCTGGTACCC TGAAAAACGAC CGAAATCTAG AGAAACACTT

3670 3680 3690 3700 3710 3720
GGAACTTAC TTCTGTGGTG TGACATAATT GGACAAACTA CCTACAGAGA TTTAAAGCTC
CCTTGAATG AAGACACCAC ACTGTATTAA CCTGTTTGAT GGATGTCTCT AAATTTTCGAG

3730 3740 3750 3760 3770 3780
TAAGGTAAAT ATAAAAATTTT TAAGTGTATA ATGTTGTTAAA CTACTGATTC TAATTTGTTG
ATTCATTTTA TATTTTAAAA ATTACATAT TACACAATTT GATGACTAAG ATTAACAAC

3790 3800 3810 3820 3830 3840
TGATATTTAG ATTCCAACTT ATGGAACCTGA TGAATGGGAG CAGTGGTGA ATGCCCTTTAA
ACATAAAATC TAAGGTTGGA TACCTTGACT ACTTACCCCTC GTCACCCACT TACGGAAATT

3850 3860 3870 3880 3890 3900
TGAGGAATAC CTGTTTGTCT CAGAAGAAAT GCCATCTAGT GATGATGAGG CTACTGCTGA
ACTCCTTTTG GACAAACGA GTCTTCTTTA CGGTAGATCA CTACTACTCC GATGACGACT

3910 3920 3930 3940 3950 3960
CTCTCAACAT TCTACTCCTC CAAAAAAGAA GAGAAAGGTA GAAGACCCCA AGGACTTTCC
GAGAGTTGTA AGATGAGGAG GTTTTTCCTT CTCTTTCCAT CTTCTGGGGT TCCTGAAAGG

3970 3980 3990 4000 4010 4020
TTTCAGAAATG CTAAGTTTGT TGAGTCAATG TGTGTTTAGT AATAGAACTC TTGCTTGCTT
AAGTCTTAAC GATTCAAAAA ACTCAGTACG ACACAAATCA TTATCTTGAG AACGAACGAA

4030 4040 4050 4060 4070 4080
TGCTATTATC ACCACAAAGG AAAAGCTGC ACTGCTATAC AAGAAATTA TGGAAAAATA
ACGATAAATG TGGTGTTCCT TTTTTCGACG TGACGATATG TTCTTTTAAT ACCTTTTAT

4090 4100 4110 4120 4130 4140
TTCTGTAAAC TTTATAAGTA GGCATAACAT TTATAATCAT AACATACTGT TTTTCTTAC
AAGACATGG AAATATTAT CATGATATGTC AATAATTAGTA TTGTATGACA AAAAAGAAATG

4150 4160 4170 4180 4190 4200
TCCACACAGG CATAGAGTGT CTGCTATTAA TAACTATGCT CAAAAATTGT GTACCTTTAG
AGGTGTCTCC GTATCTCACA GACGATAATT ATTGATACCA GTTTTAAACA CATGGAATC

A O U

FIGURE 19H

pD17-hG1b

4210 CT'TTTTAAAT TGTAAGGGG TTAATAAGGA ATATTGATG TATAGTCCT TGACTAGAGA 4260
GAAAAATTA ACATTTCCCC AATTATTCCT TATAAATAC ATATCACGA ACTGATCTCT
4270 TCATAATCAG CCATACCACA TTGTAGAGG TTTTACTTGC TTTAAAAAAC CTCCCACACC 4320
AGTATTAGTC GGTATGGTGT AAACATCTCC AAAATGAACG AAATTTTTC GAGGGTGTGG
4330 4340 4350 4360 4370 4380
TCCCCCTGAA CCTGAACAT AAAATGAATG CAATTCTTGT TGTAACTTGT TTTATTGCAG
AGGGGGACTT GGACTTTGTA TTTTACTTAC GTTAAACAACA ACAATTGAAC AANTAACGTC
4390 4400 4410 4420 4430 4440
CTTATAATGG TTACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA GCATTTTTC
GAATATTACC AATGTTTATT TCGTTATCGT AGTGTATAA GTGTTTATTT CGTAAAAAAA
4450 4460 4470 4480 4490 4500
CACTGCATTC TAGTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT GTCTGGATCG
GTGACGTAAG ATCAACACCA AACAGGTTTG AGTAGTTACA TAGAATAGTA CAGACCTAGC
4510 4520 4530 4540 4550 4560
GCTGGATGAT CCTCCAGCG GGGATCTCA TGCTGGAGTT CTTCGCCCCAC CCCAATTTGT
CGACCTACTA GGAGTCCGG CCCCTAGAGT ACGACCTCAA GAAGCGGGTG GGGTTGAACA
4570 4580 4590 4600 4610 4620
TTATTGCAGC TTATAATGGT TACAAATAAA GCAATAGCAT CACAAATTC ACAATAAAG
AATAACGTCG AATATTACCA ATGTTTATTT CGTTATCTTA GTGTTTAAAG TGT'TTATTTTC
4630 4640 4650 4660 4670 4680
CAATTTTTC ACTGCATTC AGTTGTGGTT TGTCCAAACT CATCAATGTA TCTTATCATG
GTAAAAAAG TGACGTAAGA TCAACACCAA ACAGGTTTGA GTAGTTACAT AGAATAGTAC
4690 4700 4710 4720 4730 4740
TCTGTATACC GTCGACCTCT AGCTAGAGCT TGGCGTAATC ATGGTCATAG CTGTTTCCCTG
AGACATATGG CAGCTGGAGA TCGATCTCGA ACCGCATTAG TACCAGTATC GACAAAGGAC
4750 4760 4770 4780 4790 4800
TGTGAAATGG TTATCCGCTC ACAATTCAC ACAACATACG AGCCGGAGC ATAAAGTGTA
ACACTTTTAC AATAGGCGAG TGTAAAGGTG TGT'TGTATGC TCGGCCTTCG TATTTTACAT

FIGURE 191

pD17-hG1b

4810 4820 4830 4840 4850 4860
AAGCC'NGGG TGCTAATGA G'GAGCTAAC TCACATTAAT TGCGTTGCGC TCAC'TGCCCG
TTTCGACCCC ACGGATTACT CACTCGATTG AGTGAATTA ACGCAACGCG AGTGACGGGC

4870 4880 4890 4900 4910 4920
CTTTCCAGTC GGGAAACCTG TCGTGCCAGC TGCATTAATG AATCGGCCAA CGCGCGGGGA
GAAAGGT'GAG CCTTTTGGAC AGCACGGTGC ACGTAATTAC TTAGCCGGTT GCGCGCCCTT

4930 4940 4950 4960 4970 4980
GAGGCGGT'TT GCGTATTGGG CGCTCT'TCCG CTTCCTCGCT CACTGACTCG CTGCGCTCGG
CTTCGCCANA CGCATTAACCC GCGAGAAGGC GAAGGAGCGA GTGACTGAGC GACGCGAGCC

4990 5000 5010 5020 5030 5040
TCGTTCCGGT GCGGCGAGCG GTATCAGCTC ACTCAAAGGC GGTAAATACGG TTATCCACAG
AGCAAGCCGA CGCCGCTCGC CATAGTCGAG TGAGTTTCCG CCATTATGCC AATAGGTGTC

5050 5060 5070 5080 5090 5100
AATCAGGGGA TAACGCAGGA AAGAACATGT GAGCAAAAG CCAGCAAAAG GCCAGGAACC
TTAGTCCCTT ATTGCGTCTT TTCTTGTACA CTCGTTTTTC GGTGTTTTC CCGTCTTTGG

5110 5120 5130 5140 5150 5160
GTAAAAAGGC CCGGTTGCTG GCGTTTTTCC ATAGGCTCCG CCCCCCTGAC GAGCATCACA
CAT'TTTTCCG GCGCAACGAC CGCAAAAGG TATCCGAGGC GGGGGGACTG CTCGTAGTGT

5170 5180 5190 5200 5210 5220
AAAA'CGACG C'TCAAGTCAG AGGTGGCGAA ACCCGACAGG ACTATNAAGA TACCAGGCGT
TTTT'AGCTGC GAGTTCAGTC TCCACCGCTT TGGGCTG'ACC TGATATTTCT ATGGTCCGCA

5230 5240 5250 5260 5270 5280
TTCCCCCTGG AAGCTCCC'TC GTGCGCTCTC CTGTTCCGAC CCTGCCGCTT ACCGGATACC
AAGGGGACC TTCGAGGGAG CACGGAGAG GACAAGGCTG GGACGGCGAA TGGCCTATGG

5290 5300 5310 5320 5330 5340
TGTCGCCCTT TCTCCCTTCG GGAAGCGTGG CGCTTTCTCA ATGCTCAGC TGTAGGTATC
ACAGGCGGAA AGAGGGAAGC CCTTCGCACC GCGAAGAGT TACGAGTGG ACATCCATAG

5350 5360 5370 5380 5390 5400
TCAGTTCGGT GTAGGTCTGT CGCTCCAAGC TGGGCTGTGT GCACGAACCC CCGTTCAGC
AGTCNAGCCA CATCCAGCA GCGAGGTTCG ACCCGACACA CGTGCTTTGG GGGCAAGTCG

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FIGURE 19J

pD17-hG1b

5410 5420 5430 5440 5450 5460
CCGACCGCTG CGCCTTATCC GGTAACTATC GTCTTGAGTC CAACCCGGTA AGACAGACT
GGCTGGCGAC GCGGAATAGG CCATTGATAG CAGAACTCAG GTTGGGCCAT TCTGTCTGA
5470 5480 5490 5500 5510 5520
TATCGCACT GGCAGCAGCC ACTGGTAACA GGATTAGCAG AGCAGGTAT GTAGCGGTG
ATAGCGGTGA CCGTCGTCGG TGACCATTTGT CCTAATCGTC TCGCTCCATA CATCCGCCAC
5530 5540 5550 5560 5570 5580
CTACAGAGTT CTTGAAGTGG TGGCCTAACT ACGGCTACAC TAGAAGGACA GTATTGGTA
GATGTC'CAAA GNACTTCACC ACCGGATTGA TGCCGATGTG ATCTTCCTGT CATAAACCAT
5590 5600 5610 5620 5630 5640
TCTCGCTCT GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA
AGACCGGAGA CGACTTCGGT CAATGGAAGC CTTTTCCTCA ACCATCGAGA ACTAGGCCGT
5650 5660 5670 5680 5690 5700
AACAAACCAC CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCACAGATT ACGCGCAGAA
TTGTTTGGTG GCGACCATCG CCACCAAAA AACAAACGTT CGTGTCTAA TCGCGCTCTT
5710 5720 5730 5740 5750 5760
AAAAGGATC TCAAGAAGAT CCTTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGNACG
TTTTTCTTAG AGTTCTTCTA GGAACCTAGA AAAGATGCCC CAGACTGCGA GTCACCTTGC
5770 5780 5790 5800 5810 5820
AAACTCAGC TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC
TTTTGAGTCC AATTCCCTAA AACCAGTACT CTAATAGTTT TTCCCTAGAAG TGGATCTAGG
5830 5840 5850 5860 5870 5880
TTTTAAATTA AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG
AAAATTTAAT TTTTACTTCA AAATTTAGTT AGATTTCATA TATACTCAT TGAACCCAGAC
5890 5900 5910 5920 5930 5940
ACAGTTACCA ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCTGTTTAT
TGTCATATGGT TACGAATTAG TCACTCCGTG GATAGAGTCG CTAGACAGAT AAAGCAAGTA
5950 5960 5970 5980 5990 6000
CCATAGTTCC CTGACTCCCC GTCGGTGAGA TAACTACGAT ACGGAGGGC TTACCATCTG
GGTATC'AACG GACTGAGGGG CAGCACATCT ATTGATGC'IA TGCCTC'CCCG AATGGTAGAC

FIGURE 19K

pD17-hG1b

6010 GCGCCAGTGC TGCAATGATA CCGCGAGACC CAGGCTACCC 6040 GCGTCCAGAT TTATCAGCAA 6060
CGGGGTACAG ACGTTACTAT GCGGCTCTGG GTCCGAGTGG CCGAGGTCTA AATAGTCGTT
6070 TAAACCAGCC AGCCGGAGG GCCGAGCGCA GAAAGTGTC TCGCAACTTTA TCCGCCCTCCA 6120
ATTGTGTCGG TCGGCCCTTCC CCGCTCGCGT CTTCAACCAGG ACGTTGAAAT AGGCGGAGGT
6130 TCCAGTCTAT TAATTGTTGC CCGGAAGCTA GAGTAAGTAG TTCCGCCAGTT AATAGTTTGC 6180
AGGTCAGATA ATTAACAACG GCCCTTCGAT CTCATTTCATC AAGCGGTCAA TTATCAAAACG
6190 GCAACGTTGT TGCCATTGCT ACAGGCATCG TGGTGTACG CTCGTCTGTTT GGTATGGCTT 6240
CGTTGCAACA ACGGTAACGA TGTCGGTAGC ACCACAGTGC GAGCAGCAA CCATACCGAA
6250 CATTCAGCTC CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA 6300
GTAAGTCGAG GCCAAGGTT GCTAGTTCCG CTCAAATGTAC TAGGGGGTAC AACACGTTTT
6310 AAGCGGTTAG CTCCTTTCGGT CCTCCGATCG TTGTCAGAAG TAAAGTTGGC GCAGTGTATT 6360
TTCCGCCAATC GAGGAAGCCA GGAGGCTAGC AACAGTCTC ATTCAACCGG CGTCACAATA
6370 CACTCATGGT TATGGCAGCA CTGCATTAAT CTCTTACTGT CATGCCATCC GTAAGATGCT 6420
GTGAGTACCA ATACCGTCTG GACGTATTAA GAGAATGACA GTACGGTAGG CATTCACGA
6430 TTTCCTGTAC TGGTGAGTAC TCAACCAAGT CATTCTGAGA ATAGTGTATG CCGCGACCGA 6480
AAAGACACTG ACCACTCATG AGTTGGTTCA GTAAGACTCT TATCACATAC GCCGCTGGCT
6490 GTTGTCTTGT CCGGCGTCA ATACGGGATA ATACCGCGCC ACATAGCAGA ACTTTAAAAG 6540
CAACGAGAAC GGGCCGCAGT TATGCCCTAT TATGGCGCGG TGTATCGTCT TGAATATTTTC
6550 TGCTCATCAT TGGAAAACGT TCTTCGGGGC GAAACTCTC AAGGATCTTA CCGCTGTTGA 6600
ACGAGTAGTA ACCTTTGTGA AGAAGCCCGG CTCTTTGAGAG TTCTTAGAAT GCGGACAACT

FIGURE 19L

pD17-hG1b

6610	6620	6630	6640	6650	6660
GATCCAGTTC	GATGTAACCC	ACTCGTGAC	CCAACTGATC	TTCAGCATCT	TTTACTTTTCA
CTAGGTCAAG	CTACATTGGG	TGAGCACGTG	GGTTGACTAG	AAGTCGTAGA	AAATGAAGT
6670	6680	6690	6700	6710	6720
CCAGCGTTC	TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAG	GGAAATAAGG
GGTCGCAAG	ACCCACTCGT	TTTTGTCCTT	CCGTTTACG	GGTTTTTTC	CCTTATTCCC
6730	6740	6750	6760	6770	6780
CGACACGGAA	ATGTTGAATA	CTCATACTCT	TCCTTTTTC	ATATTATTGA	AGCATTTATC
GCTGTGCCCT	TACAACTTAT	GAGTATGAGA	AGGAAAAAGT	TATAATTA	TCGTAAATAG
6790	6800	6810	6820	6830	6840
AGGGTTATTG	TCCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG
TCCCAATAAC	AGAGTACTCG	CCTATGTATA	AACTTACATA	AATCTTTT	TTTGTTTATC
6850	6860	6870	6880	6890	6900
GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CGACGGATCG	GGAGATCTGC
CCCAAGGCGC	GTGTAAAGCG	GCTTTTCACG	GTGGACTGCA	GCTGCCCTAGC	CCTCTAGACG
6910	6920	6930	6940	6950	6960
TAGGTGACCT	GAGGCGCGCC	GGCTTCGAAT	AGCCAGAGTA	ACCTTTT	TTAATTTTAT
ATCCACTGGA	CTCCGCGCGG	CCGAAGCTTA	TCGGTCTCAT	TGGAAAAAAA	AATTAAAAATA
6970	6980	6990	7000	7010	7020
TTTATTATTAT	TTTGTGAGATG	GAGTTTGGCG	CCGATCTCCC	GATCCCCCTAT	GGTCGACTCT
AAATAAAATA	AAACTCTAC	CTCAAAACCGC	GGCTAGAGGG	CTAGGGGATA	CCAGCTGAGA
7030	7040	7050	7060	7070	7080
CAGTACAATC	TGCTCTGATG	CCGCATAGTT	AAGCCAGTAT	CTGCTCCCTG	CTTGTTGTGT
GTCTATGTTAG	ACGAGACTAC	GGCGTATCAA	TTCCGTCATA	GACGAGGGAC	GAACACACAA
7090	7100	7110	7120	7130	7140
GGAGTCCGCT	GAGTAGTGCG	CGAGCAAAAT	TTAAGCTACA	ACAAGGCAAG	GCTTGACCGA
CCTCCACGCA	CTCATCACGC	GCTCGTTT	AATTCGATGT	TGTTCCGTTT	CGAAGCTGCT
7150	7160	7170	7180	7190	7200
CAATTCATG	AAGAACTCTG	TTAGGGTTAG	GCGTTTTCG	CTGCTTCGCG	ATGTACGGGC
GTTAACCTAC	TCTTTAGACG	AATCCCAATC	CGCAAAACGC	GACGAAGCGC	TACATGCCCG

FIGURE 19M

pD17-hG1b									
7210	7220	7230	7240	7250	7260				
CAGATATACG	CGTGTACATT	GATTATTGAC	TAGTTATTAA	TAGTAATCAA	TTACGGGGTC				
GTCTATATGC	GCAACTGTAA	CTAATAACTG	ATCAATAAAT	ATCATTAGTT	AATGCCCCAG				
7270	7280	7290	7300	7310	7320				
ATTAGTTTAT	AGCCCATATA	TGGAGTTCCG	CGTTACATAA	CYTACCGTAA	ATGGCCCGCC				
TAATCAAGTA	TCCGGGTATAT	ACCTCAAGGC	GCAATGTATT	GAATGCCATT	TACCGGGCGG				
7330	7340	7350	7360	7370	7380				
TGGCTGACCG	CCCAACGACC	CCCGCCCAAT	GACGTCAATA	ATGACGTATG	TTCCCATAGT				
ACCGACTGGC	GGGTTCCTGG	GGGCGGGTAA	CTGCAGTTAT	TACTGCATAC	AAGGGTATCA				
7390	7400	7410	7420	7430	7440				
AACGCCAATA	GGGACTTTCC	ATTGACGTCA	ATGGGTGGAC	TATTACGGT	AAACTGCCCA				
TTGGCGTTAT	CCCTGAAGG	TAACTGCAGT	TACCCACCTG	ATAAATGCCA	TTTGACGGGT				
7450	7460	7470	7480	7490	7500				
CTTGGCAGTA	CATCAAGTGT	ATCATAATGC	AAGTACGCCC	CCTATTGACG	TCAATGACGG				
GAACCGTCAT	GTAGTTTACA	TAGTATACGG	TTCATGCGGG	GGATAACTGC	AGTTACTGCC				
7510	7520	7530	7540	7550	7560				
TAAATGGCCC	GCCTGGCATT	ATGCCCAAGTA	CATGACCCTTA	TGGGACTTTC	CTACTTGGCA				
ATTTACCGGG	CGGACCGTAA	TACGGGTCTAT	GTACTGGAAT	ACCCTGAAAG	GATGAACCGT				
7570	7580	7590	7600	7610	7620				
GTACAATCTAC	GTAATTAGTCA	TCGCTATTAC	CATGGTGATG	CGGTTTGGC	AGTACATCAA				
CATGTAGATG	CATAATCAGT	AGCGATAATG	GTACCACCTAC	GCCAAAACCG	TCATGTAGTT				
7630	7640	7650	7660	7670	7680				
TGGGCGTGGA	TAGCGGTTTG	ACTCACGGGG	ATTTCCAAAGT	CTCCACCCCA	TTGACGTCAA				
ACCCGCACCT	ATCGCCAAAC	TGAGTGCACC	TAAAGGTCCA	GAGGTGGGGT	AACTGCAGTT				
7690	7700	7710	7720	7730	7740				
TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA	ACAACTCCGC				
ACCCCTCAAC	AAAACCGTGG	TTTTAGTTGC	CCTGAAAGGT	TTTACAGCAT	TGTTGAGGCG				
7750	7760	7770	7780	7790	7800				
CCCATTTGACG	CAANTGGGCG	GTAGGCGTGT	ACGGTGGGAG	GTCTATATAA	GCAGAGCTCT				
GGGTAACCTGC	GTTTACCCCGC	CATCCGCACA	TGCCACCCCTC	CAGATATATT	CGTCTCGAGA				

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FIGURE 19N

pD17-hG1b

7810	CTGGCTAACT	7820	AGAGAACCCA	7830	CTGCTTACTG	7840	GCTTATCGAA	7850	ATTAAATACGA	7860	CTCACTATAG
	GACCGATTGA		TCTCTTGGGT		GACGAATGAC		CGAATAGCTT		TAATTATGCT		GAGTGATATC
7870	GGAGACCCAA	7880	GCTT								
	CCTCTGGGTT		CGAA								

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FIGURE 20

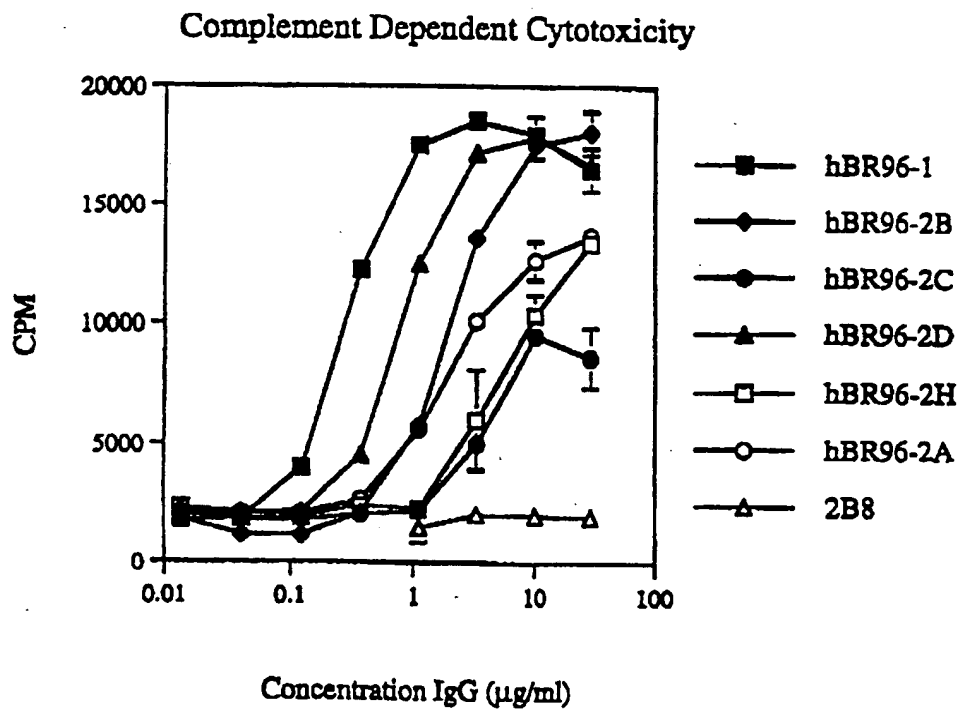


FIGURE 21

Antibody Dependent Cell-Mediated Cytotoxicity

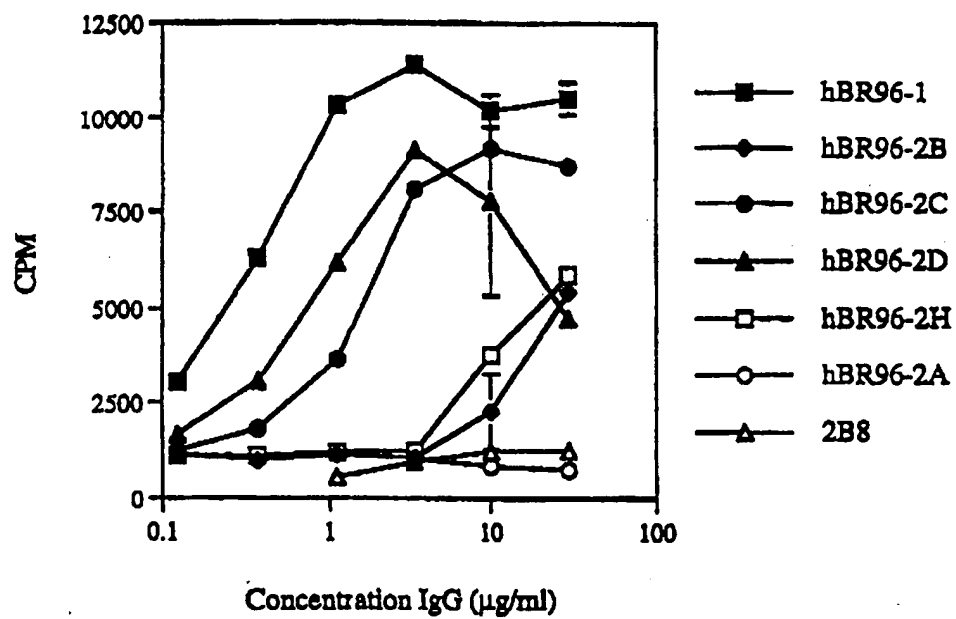


FIGURE 22

Binding activity of hBR96-2 constant region mutants on L α -HSA

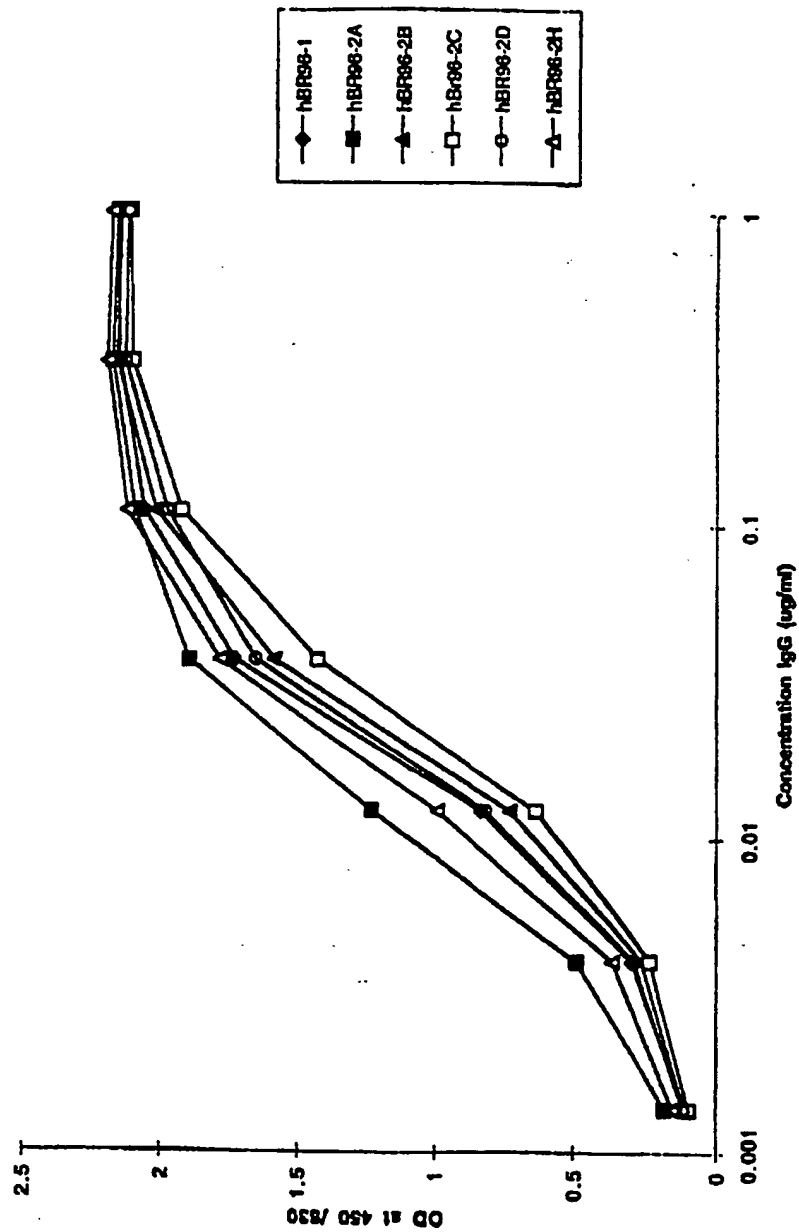
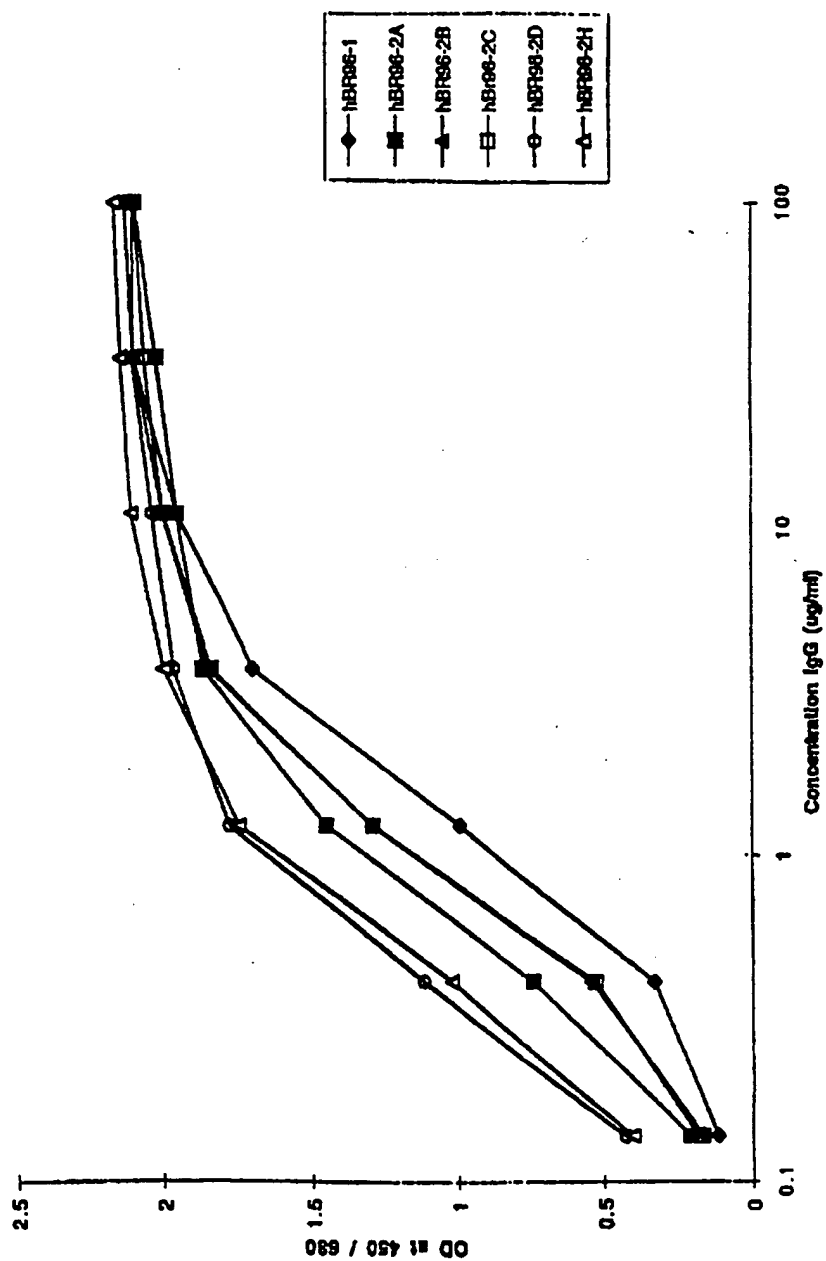


FIGURE 23

Binding activity of hBR96-2 constant region mutants on LNFPII-BSA



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Figure 24

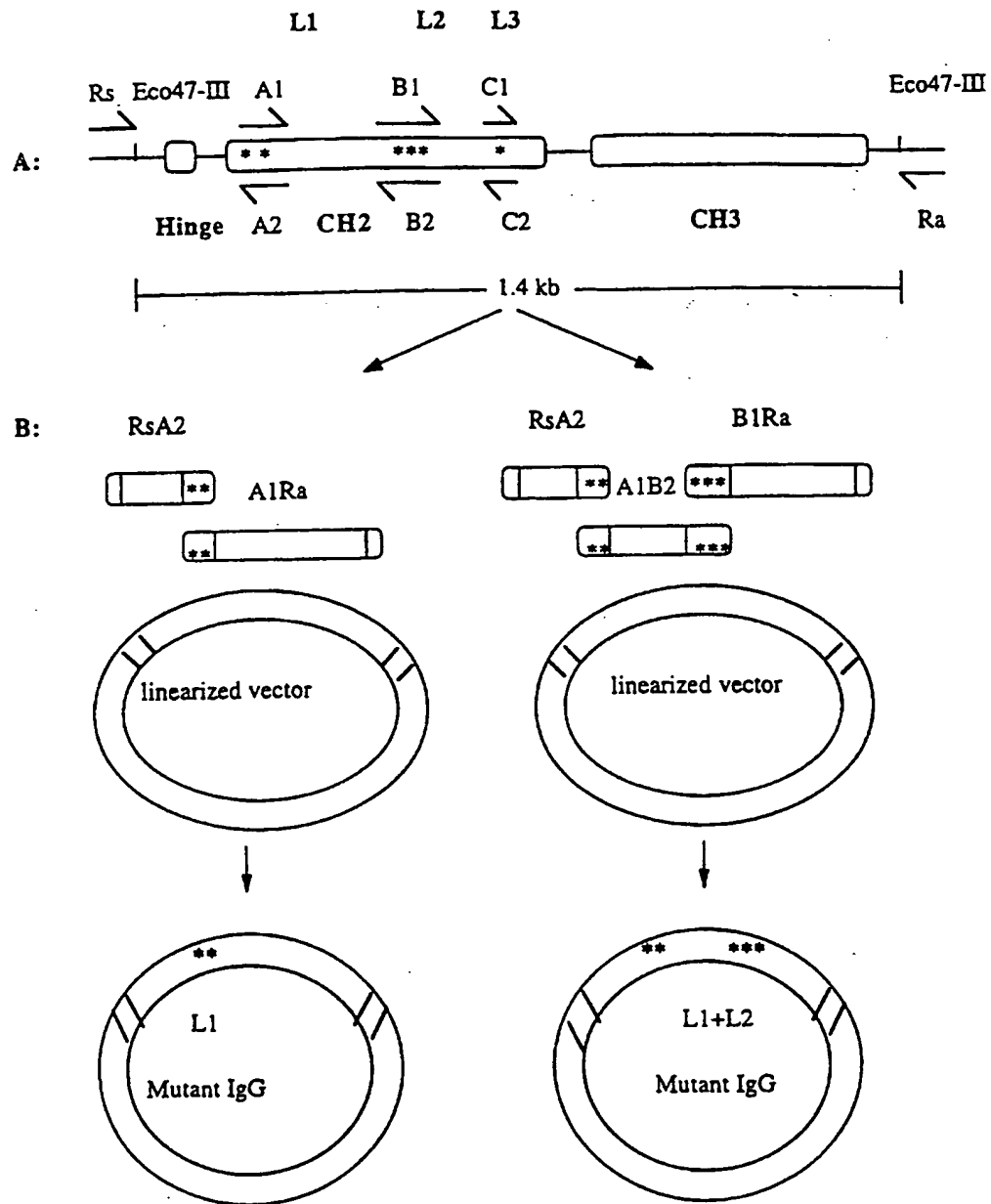


Figure 25

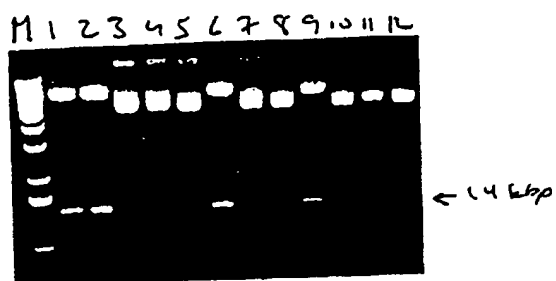


Figure 26

hBR96-2 Heavy Chain Variable Region (VH)

1 11 21 31 41
 EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY
 51 61 71 81 91
 ISQDGDITDY ADSVKORFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
 101 111
 ADGAWFAYWG QGTLVTSS

human IgG1 constant

121
 A STKGPSVFPL APSSKSTSGG TAALGCLVKD
 YFPEPVTFSW NSGALTSGVH TFPVQLQSSG LYSLSSTVTV PSSSLGTQTY
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 ICN1300

Figure 27

hBR96-2A: Heavy Chain Variable Region (VH)

1 11 21 31 41
EVQLVESGGG LVQPGGSLRL SCAASGFPFS DYYMYWVRQA PGKGLEWVSY
51 61 71 81 91
ISQDGDITDY ADSVKGRFTI SRDNAKNSLY LQMNSLRDED TAVYYCARGL
101 111
ADGAWFAYWG QGTLTVSS

hBR96-2A: Human Heavy Chain IgG1 Constant Region Δ CH2

A STKGPSVFPL APSSKSTSCG TAALGCLVKD YFPEPVTVSW NSGALTSGVH
TFFAVLQSSG LYSLSVVTV PSSSLGTQTY ICNVNKKPSN TKVDKKVEPK
SCDKTHTCPP CP CQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA
VEWESNGQPE NNYKTTTPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVN
HEALHNYTQ KSLSLSPGK

Figure 28

This sequence is the chi BR96 IgG1 with CH2 deleted.

1 ^{VH} EVNLVESGGG LVQPGGSLKV SCVTSGFTFS DYMYWVRQT PEKRLWVAY
51 ISQGGDITDY PDTVKGRPTI SRDRAKNTLY LQMSRLKSED TAMYFCARGL
101 DDGAWFAYWG QGTLVTVSVA ^{CH1} STKGPSVFPL APSSKSTSGG TAALGCLVKD
151 YFPEPVTVSW NSGALTSGVH TFPVLQSSG LYSLSVVTV PSSSLGTQTY
201 ICNVNKKPSN TKVDKKVEPK SCDKTHTCP ^{CH3} CHGQPREPQV YTLPPSRDEL
251 TRNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTTPVL DSDGSFFLYS
301 KLTVDKSRWQ QGNVFSCSVN HEALHNHYTQ KSLSLSPGK

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/13562

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/62 A61K39/395 A61K38/17 A61K47/48 A61K51/10
C07K16/30 C07K16/46 C07K16/00 C12N15/13 C12N1/21
C12N5/10 //C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. GILLIES ET AL.: "Antigen binding and biological activities of engineered mutant chimeric antibodies with human tumor specificities." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 1, no. 1, 1990, STONEHAM, MA, USA, pages 47-54, XP002050448 see the whole document --- -/-	1-8, 23-25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

17 December 1997

Date of mailing of the international search report

21.01.98

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Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

Internat Application No
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. SCHREIBER ET AL.: "An unmodified anticarcinoma antibody, BR96, localizes to and inhibits the outgrowth of human tumors in nude mice." CANCER RESEARCH, vol. 52, no. 12, 15 June 1992, BALTIMORE, MD, USA, pages 3262-3266, XP002050449 see abstract	33,35,36
A	---	1,2,5,7, 8,11-18, 23
A	A. DUNCAN ET AL.: "The binding site for Clq on IgG." NATURE, vol. 332, no. 6166, 21 April 1988, LONDON, GB, pages 738-740, XP002050450 cited in the application see the whole document	1,2,5,7, 8
A	---	1,2,5,7, 8
A	J. LUND ET AL.: "Human FcγRI and FcγRII interact with distinct but overlapping sites on human IgG." THE JOURNAL OF IMMUNOLOGY, vol. 147, no. 8, 15 October 1991, BALTIMORE, MD, USA, pages 2657-2662, XP002050451 cited in the application see abstract	1,2,5,7, 8
A	---	1-8
A	Y. XU ET AL.: "Residue at position 331 in the IgG1 and IgG4 CH2 domains contributes to their differential ability to bind and activate complement." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 5, 4 February 1994, BALTIMORE, MD, USA, pages 3469-3474, XP002050452 cited in the application see abstract see discussion	1-8
A	---	1,2,5,7, 8
	T. MICHAELSEN ET AL.: "One disulfide bond in front of the second heavy chain constant region is necessary and sufficient for effector functions of human IgG3 without a genetic hinge." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 91, no. 20, 27 September 1994, WASHINGTON, DC, USA, pages 9243-9247, XP002050453 see the whole document	1,2,5,7, 8

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INTERNATIONAL SEARCH REPORT

Intern. Appl. Application No.
PCT/US 97/13562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	L. TAN ET AL.: "Influence of the hinge region on complement activation, C1q binding, and segmental flexibility in chimeric human immunoglobulins." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 87, no. 1, January 1990, WASHINGTON, DC, USA, pages 162-166, XP002050454 see the whole document ---	1-8
A	EP 0 699 756 A (BRISTOL-MYERS SQUIBB COMPANY) 6 March 1996 cited in the application see examples see claims -----	11-18, 23,25, 28,29, 31-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/13562

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 97/13562

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 26,27

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claim 26 represents a method of detection/diagnosis and refers forward to claim 30, which represents a method of treatment. Claim 27 refers to a method in claim 24; however, in claim 24 a product is claimed, not a method.

Remark : Although claims 1-22, 25, 28-32 and 34-36 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

information on patent family members

PCT/US 97/13562

Form PCT/ISA/210 (patent family annex) (July 1992)